The Operation of a Hospital Transfusion Service

PART I—The Preservation and Transfusion of Whole Human Blood

PART II—The Processing and Use of Citrated Human Blood Plasma

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Foreword

This manual describes some of the techniques which have proved satisfactory for the operation of hospital blood banks and for the preparation of human plasma. They are based upon the experience of many investigators and have the approval of the Subcommittee on Blood Substitutes of the Division of Medical Sciences, National Research Council.

The establishment of blood and plasma banks, particularly in large hospitals, has been of inestimable value in saving the lives of patients suffering from shock and burns, in improving the care of surgical patients, and in providing a valuable adjunct in the treatment of certain medical conditions. Although they are designed primarily to supply the normal civilian needs of the hospital, they may be rapidly expanded to serve practically any major catastrophe. Unlike many establishments which are necessary in time of war but become obsolescent as soon as peace is declared, the blood bank will continue to be useful.

The fact that plasma and other blood derivatives are lifesaving in emergencies must not lead to the conclusion that such substances can completely replace whole blood transfusions. Although this was never the original intention of the advocates of blood derivatives, the trend has been to regard plasma, serum, etc., as "blood substitutes" rather than blood derivatives, which serve a specific purpose but cannot replace whole blood. In nearly all emergency cases in which trauma (or surgery) is associated with significant blood loss, the transfusion of whole blood is preferable to the use of plasma, provided that it is *immediately* available. If whole blood cannot be administered *at once*, plasma (serum, etc.) must be given *promptly* to meet the emergency and followed later by whole blood as indicated.

In addition, there is growing interest in the possible uses of convalescent plasma (or serum) in the treatment of infectious diseases. The ability of a hospital to prepare and store plasma in the frozen state will enable its staff to make use of the developments in this field.

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Part I. The Preservation and Transfusion of Whole Human Blood

A. CLINICAL AND EXPERIMENTAL BACKGROUND

Chapter I. *Blood Groups: Isohemagglutinins and Isohemolysins*

Nomenclatures for Blood Groups

In 1907 Jansky suggested the designation of the blood groups by numbers, and a short time later Moss made a similar proposal, but he reversed the Jansky groups I and IV. The confusion between the numberings by Jansky and by Moss is now eliminated by the use of the system called the International Nomenclature. The use of numbers for the designation of blood groups is to be discouraged. Table 1 shows the relationship between the various nomenclatures.

Table 1

Approximate distribution among white individuals in U. S. A.

International	Jansky	Moss	Percent
O	I	IV	44
A	II	II	38
B	III	III	14
AB	IV	I	4

All human bloods are divided into four groups according to the agglutinogens contained in the cells. The two agglutinogens A and B may be distributed in four ways as shown in table 2. The four blood groups are therefore known as A, B, AB, and O in the preferred International Nomenclature. The serum or plasma may contain specific agglutinins, each of which reacts against only the A or B agglutinogen. The combination of cells containing agglutinogen A with serum or plasma containing anti-A agglutinin will produce permanent clumping or agglutination of the cells. It is obvious, therefore, that no single blood can contain an agglutinogen and its corresponding agglutinin. Group A blood contains anti-B agglutinin. Group B

blood possesses anti-A agglutinin. Group O blood contains both anti-A and anti-B, and AB blood contains no agglutinins. (See table 2). About 30 percent of fresh bloods having agglutinins contain hemolysins which are specific and react against the same agglutinogen as the agglutinin. The hemolysins are weaker in titer than the agglutinins and will act only in the presence of complement. They are therefore not encountered when typing serum is used, but their presence constitutes a source of error in the cross-matching of bloods.

Table 2

Group	Agglutinogens in cells	Agglutinins in serum or plasma
O	None	Anti-A and anti-B
A	A	Anti-B
B	B	Anti-A
AB	A and B	None

Inheritance

The individual inherits the combination of group-specific substances which determines his blood group. It follows that the blood group cannot change during the life of the individual. According to Bernsteinin, there are two laws governing the inheritance of blood groups: 1. Agglutinogens A or B do not appear in the blood of the child except when present in one or both of the parents. 2. The combinations, group O parents and AB child, or vice versa, are impossible.

The Changes in Titer of Agglutinins and Agglutinogens During Life

This is of importance in the cross-matching and typing of blood. Agglutinogens appear as early as the thirty-seventh day of fetal life. The titer increases until the twentieth year, after which it remains constant. The erythrocytes of a new-born baby are only one-fifth as sensitive to agglutination as are those of an adult. There is some doubt as to whether agglutinins are present at birth. At any rate, the titer quickly increases and reaches a maximum between 5 and 10 years, after which there is a slow diminution of potency. These facts account for the difficulty sometimes encountered in determining the blood groups of children.

Neutralization of Agglutinins in the Body

When an agglutinin and a group-specific substance come in contact, a combination is effected which is permanent and results in the neutralization of the agglutinin. This phenomenon occurs when incompatible agglutinins are injected into the body, or when group O blood

is transfused into a recipient of another group. This fact explains the safety with which pooled plasma is employed (31), but there is still some question as to whether the use of blood from a group O or "universal donor" is entirely innocuous. There are many who believe that the use of the universal donor is without danger and attribute the cases of reactions reported in the literature to some other cause. The Rh factor, for example, has only recently been recognized, and the older studies did not consider this possibility. One case has recently been reported, however, in which the anti-Rh factor was definitely excluded (27).

The Neutralization of the Agglutinins in Vitro

This has been made practical by the preparation of the group-specific A and B substances (agglutinogens) by Witebsky et al. (49). These substances have been prepared in a form safe for intravenous injection. The addition of small amounts to group O blood immediately inactivates the anti-A and anti-B agglutinins in the plasma, thus producing a universal blood which is free from theoretical objection.

The Rh Antibody

The red blood cells of approximately 85 percent of all persons, irrespective of their blood groups, contain an agglutinogen designated Rh, related to a similar agglutinogen found in the red cells of rhesus monkeys. Apparently there are no naturally occurring anti-Rh agglutinins, but the Rh negative individuals (15 percent of the population) are capable of forming anti-Rh agglutinins. This may occur (1) when repeated transfusions of Rh positive blood are given to an Rh negative subject, or (2) when an Rh negative woman bears an Rh positive fetus (from an Rh positive father). Not all Rh negative individuals develop demonstrable isoagglutinins under these circumstances, but following such isoimmunization, the Rh negative individual may suffer severe hemolytic reactions when transfused with Rh positive blood cells. Manifestations of Rh isoimmunization usually do not appear until after several transfusions (a variable number), when reactions, usually mild at first, may occur and increase in severity following each succeeding transfusion with Rh positive blood. Manifestations of Rh isoimmunization usually do not occur in the first pregnancy of the Rh negative woman. However, in subsequent pregnancies, the anti-Rh agglutinins increasingly formed by the mother cause in her Rh positive infants a profound hemolytic anemia known as erythroblastosis fetalis.

If a patient receiving repeated blood transfusions has had reactions to previous transfusions, it is important to rule out Rh isoimmunization as a cause of the reactions by Rh typing of the recipient and donor

Should any woman with a history of having borne an erythroblastotic infant require a transfusion, Rh typing of recipient and donor should be done before any transfusion, because in such instances even a first transfusion may provoke a fatal reaction. In such cases Rh negative blood of a compatible group can be given safely. Only such blood should be given in the treatment of infants with erythroblastosis, since anti-Rh agglutinins from the mother's blood are present in their circulation for some time. (*See page 23 for avoiding Rh incompatibility by special typing or cross-matching.*)

Chapter II. *The Donor*

Physical Standards

It is the general practice to accept as blood donors both men and women who:

1. Assert they are in good health.
2. Are between the ages of 18 and 60.
3. Give no history of:
   a. Recent asthma. (It is apparently not dangerous to transfuse the blood of a donor having pollen sensitivity outside of the pollen season.)
   b. Repeated attacks of angioneurotic edema.
   c. Malaria. (Need not disqualify for preparation of plasma which will be frozen or dried.)
   d. Recent chancre or positive serologic tests for syphilis.
   e. Jaundice, occurring within the previous 6 months. (This precaution is thought to eliminate the possibility of the transmission of infectious jaundice by transfusion.)
4. Do not show the following physical or laboratory findings:
   a. Evidence of chancre or positive serologic tests for syphilis. (Inspection of the genitalia should be compulsory in male donors.)
   b. Elevation of temperature over 99.5° F. by mouth.
   c. Systolic blood pressure of over 200 mm. Hg. or diastolic of over 120. (If a vascular accident occurred following blood donation, it might be ascribed to the donation of blood.)
   d. Systolic blood pressure of less than 100 mm. Hg. (Donor reactions very common in this group.)
   e. Hemoglobin less than 80 percent of normal.

Discussion.—In ascertaining that the donor's health is good, it is advisable to inquire into the presence of a cold or sore throat, any recent illness, any chronic illness (particularly heart disease, pulmonary tuberculosis, or diabetes), and symptoms such as shortness of breath, persistent cough, chest pain, etc. A positive answer to one or more of these questions would necessitate further investigation and perhaps consultation with the prospective donor's physician before acceptance of the person. A history of pulmonary tuberculosis or the existence of diabetes should disqualify a donor unless he presents a written statement from his physician that he may donate his blood. It has been noted, also, that donors with a history of frequent fainting or convulsions are very prone to develop untoward reactions when they give blood. Legally, persons under 21 years of age should be required to present written parental permission before being accepted as blood donors.

Physiology of Controlled Hemorrhage

Recent physiologic studies (21, 45) have thrown much light on the readjustments which take place in the body after controlled hemorrhage, such as the donor undergoes. After the withdrawal of from 500 to 1,200 cc. of blood, the pulse rate may either remain normal or be increased, or bradycardia may actually develop. The blood pressure frequently falls to levels of 80 or 90 mm. systolic. Some persons show an exaggerated response to changes in posture, the systolic pressure falling instead of rising when the erect position is resumed. This condition may obtain for hours and probably accounts for some of the syncopal attacks which are sometimes encountered when the donor gets off the bleeding table. In bleedings of from 500 to 1,200 cc. it takes between 48 and 72 hours for the blood volume to be restored to normal. During this interval there is a steady increase in the fluid content of the blood, but for the first 2-hour period the added fluid is extremely poor in protein. Thereafter, the fluid has a protein concentration similar to that of normal plasma.

In one study (23) it took about 50 days for males to regenerate the hemoglobin lost in donating blood for one transfusion; females took slightly longer. This interval was shortened to 35 days by the daily administration of 1 gram of iron and ammonium citrate. It is a safe rule that *not more than 500 cc. of blood be withdrawn every 90 days* from donors who are not carefully supervised.

Chapter III. *The Recipient*

Indications for Transfusion

The principal functions of whole blood and plasma transfusions may be classified as shown:

Table 3

Indication	Whole blood		Plasma or serum	
	Choice	State (fresh or preserved)	Choice	State (fresh liquid, stored liquid, frozen, dried)
Shock due to hemorrhage (traumatic shock).	First[1]	No preference	Second	No preference.
Shock with hemoconcentration (burns, crush syndrome).	Second	No preference	First	No preference.
Hypoproteinemia	Second	No preference	First	No preference.
Acute and chronic anemias	Imperative	No preference	Not indicated.	
CO poisoning and methemoglobinemia.	Imperative	No preference	Not indicated.	
Immune therapy	Second	No preference	First	Fresh liquid, frozen or dried.
Deficiency of complement	Either	Fresh	Either	Fresh liquid, frozen or dried.
Deficiency of prothrombin	Either	Fresh	Either	Fresh liquid, frozen or dried.
Leukopenia and thrombocytopenia.	Imperative	Fresh	Not indicated.	
Hemophilia	First	Fresh	Second	Fresh liquid, frozen or dried.

[1] The recommendation of first and second choice is made on the assumption that both blood and plasma are immediately available

Contra-indications

There are few contra-indications for blood or plasma transfusion, but these should be observed with extreme care. The presence of edema of the lungs due to cardiac decompensation is almost always a contra-indication. It has been shown that failure of the left side of the heart may be produced by the intravenous injection of as little as 200 cc. of saline in an individual with borderline compensation (38). In extreme cases edema of the lungs may be produced by the rapid injection of as little as 50 cc. of blood.

Survival of Transfused Erythrocytes

Of prime interest in blood transfusion is the time of survival of the transfused erythrocytes. The data for fresh blood have shown considerable variation due to the different methods of storage employed. In general, comparisons between fresh and preserved blood are more informative because the same methods have been used throughout. By means of the differential agglutination method of Ashby, it has been shown (7) that blood stored in sodium citrate for 8 days survives in the recipient approximately one-half as long as fresh blood. Blood stored in dextrose-citrate solutions survives for much longer periods (7, 33, 34). When the concentration of dextrose is high, as in the Rous-Turner mixture, 42 percent of the cells stored for 28 days were present in the circulation 1 month after transfusion (35). The survival period of blood stored in dextrose-citrate solutions has recently been confirmed by Denstedt (56). Clinical experience with dextrose preserved blood in a large number of hospitals has been entirely satisfactory.

Chapter IV. *Transfusion Reactions*

It is of the utmost importance to attempt the differentiation of the various types of transfusion reactions, since the etiology of each type is different. It is no more diagnostic to state unqualifiedly that "the patient had a transfusion reaction" than to state that "the patient had a fever." Transfusion reactions are frequently difficult to differentiate from the manifestations of the underlying disease. The following categories include the great majority of transfusion reactions.

Pyrogenic Reactions

Clinical description.—While the transfusion is being received or soon thereafter, the patient may experience a chill. This is usually followed by a rise in temperature to 101° or 102° F. The fever persists for 5 or 6 hours and subsides without treatment. Occasionally there is a definite chill with no elevation of temperature, or there may be no chill preceding the fever.

Etiology.—Certain water-borne bacteria may grow in distilled water and give off soluble ultrafilterable products which are not inactivated by temperatures usually used in sterilization. When these substances, which are called pyrogens, are injected intravenously they produce the clinical syndrome described above. Lack of care in the preparation of blood transfusion equipment and of the fluids employed

is undoubtedly responsible for most of the pyrogenic reactions accompanying transfusion.

Prophylaxis.—Proper preparation of transfusion equipment and fluids for intravenous therapy; avoid contamination.

Treatment.—Mild reactions require no treatment. If reactions are severe, the transfusion should be discontinued.

Different diagnosis.—Onset with severe chills and fever with some dyspnea suggest the possibility of the much graver hemolytic type of reaction. The transfusion should be promptly discontinued, and the typing and cross-matching should be rechecked at once (see "Hemolytic Reactions").

Urticarial Reactions

Clinical description.—At any time during the course of the transfusion or immediately afterward the patient may develop an urticarial eruption which may consist of only a few "hives," or may become generalized. In some individuals the process takes the form of angioneurotic edema, in which one part of the body becomes massively edematous. The periorbital tissue and the lips are most commonly involved in this process. The eruption usually subsides in from 6 to 24 hours. There is some danger that in the severe cases edema of the larynx may develop.

Etiology.—Little is known about the cause of many of these reactions. It has been observed that the blood from an individual with repeated attacks of angioneurotic edema has produced similar manifestations in recipients. On the other hand, in many instances neither donor nor recipient gives a history of allergic manifestations. A recipient may react to a certain blood with the first injection and not with subsequent injections. The same blood may produce urticaria in one individual and not in another.

Prophylaxis.—A small proportion of reactions may be prevented by disqualifying donors who are subject to angioneurotic edema. The use of blood from fasting donors will tend to diminish this type of reaction.

Treatment.—With minor degrees of eruption, the transfusion may be continued, but severe manifestations require discontinuance. The drug of choice is epinephrin hydrochloride, 0.5 cc. (1–1000 solution) subcutaneously.

Differential diagnosis.—This offers no difficulty.

Hemolytic Reactions

Clinical description.—A distinction must be made between the symptoms following the intravenous injection of free hemoglobin and the symptoms occurring after intravascular hemolysis. This probably is largely a matter of the amount of hemoglobin involved, since

it is possible to release much greater amounts of free hemoglobin by the rupture of cells already in the circulation than can be introduced through a needle in unit time. The intravenous injection of small amounts of hemoglobin causes no symptoms, but when large amounts are given, the patient complains of a feeling of constriction behind the sternum, chilliness, fever and pain in the lumbar region (24). The symptoms are similar when slight degrees of hemolysis have occurred intravascularly. When red cell destruction has been more extensive, the patient may go into shock, with cold, clammy skin, lowered arterial tension, and air hunger. Death may occur at this stage if proper treatment for shock is not immediately instituted.

If shock does not appear, or is successfully overcome, another manifestation to be expected is jaundice. This has been observed within six hours after an accident. Blood bilirubin values of from 10 to 20 mg. percent are not uncommon. The blood serum may be colored red by free hemoglobin for only a few hours and turn yellow as the hemoglobin is converted into bilirubin. The urine may contain sufficient hemoglobin to appear bright red. On standing it becomes brown. The hemoglobinuria may disappear within 48 hours and recovery be uneventful. The jaundice fades within a few days. In the exceptional case, however, oliguria or anuria develops, usually during the first 24 hours. This results in progressive azotemia. The lowering of the alkali reserve due to the development of acidosis is a characteristic feature. Coma supervenes during the last few days of life. Death from uremia usually results on the fourth to nineteenth day after transfusion unless spontaneous diuresis occurs, in which case complete recovery is the rule. Hypertension may develop.

Patients dying of hemolytic transfusion reaction show a characteristic pathologic picture. There are no gross lesions except some edema of the parenchyma of the kidneys. Microscopically there is interstitial edema of the kidneys with varying degrees of tubular necrosis. Tubular regeneration may be evidenced by an increased number of mitotic figures. The glomerular tufts are normal, and the lumina of the proximal convoluted tubules are usually dilated. The distal convoluted tubules may contain pus cells, cellular debris, and a few casts of yellowish brown pigment, which is thought to be hemoglobin. The interstitial tissue is frequently infiltrated with polymorphonuclear leukocytes. In some cases there are areas of focal necrosis in the liver (9, 10, 11, 12, 26).

Etiology.—The most common cause of intravascular hemolysis is the transfusion of incompatible blood. Experience indicates that the transfusion of 75 to 100 cc. of such blood is necessary to cause a severe reaction. Other causes of hemolysis should also be considered. The blood may have been hemolyzed before injection into the body as a

result of improper storage, freezing, application of excessive heat, or the addition of distilled water or other hemolytic agents. Intravascular hemolysis may occur spontaneously in malaria, paroxysmal hemoglobinuria, and idiosyncrasy to quinine and sulfonamides.

There is a renal threshold for hemoglobin (24, 39). In normal kidneys hemoglobin appears in the urine when the plasma level reaches about 135 mg. per 100 cc. The threshold is lower in damaged kidneys. Empirically, it has proved a safe rule that injections of free hemoglobin insufficient to exceed the renal threshold are innocuous.

Prophylaxis.—The prevention of hemolysis lies in an understanding and avoidance of the factors causing it, as outlined under etiology, and in the care with which the recipient is transfused. The results of some animal studies (10, 11) suggest that routine alkalinization of the urine before transfusion might prevent transfusion anuria. This, however, is not at all certain.

Treatment.—If the patient with a hemolytic reaction develops neither shock nor anuria, recovery is spontaneous. If shock develops, prompt transfusion with plasma may be indicated. The immediate administration of alkalis is indicated to forestall or minimize the precipitation of hematin in the renal tubules. If, however, renal insufficiency develops, every effort should be made to reestablish an adequate urinary output.

Differential diagnosis.—If the patient is seen within a few hours after receiving the transfusion, a specimen of his blood should be immediately withdrawn in dry, clean apparatus and centrifuged. The presence of hemoglobin in the supernatant fluid will indicate hemolysis. In the examination of the urine it is important to differentiate between hematuria and hemoglobinuria. Hematuria is not evidence of a blood transfusion reaction.

Cardiovascular Reactions

Clinical description.—This complication usually occurs in patients suffering from chronic cardiac disease. During or immediately after transfusion, the recipient may become extremely dyspneic and cyanotic. Fine crackling and coarse rales may be heard in the lungs. The patient may recover spontaneously or die within a few hours. At autopsy the lungs are edematous, and there may be evidence of cardiac dilatation.

Etiology.—It is now recognized that even small increases in the blood volume of patients with borderline cardiac compensation may result in left ventricular failure. Frank cardiac decompensation is usually easily recognized, and such patients should almost never be transfused. It is in the unrecognized borderline cases that transfusions are most likely to produce circulatory embarrassment. Death has been reported (12) from the injection of 200 cc. of blood into the adult

recipient. However, it should be borne in mind that cardiac patients who have been severely injured or burned may require transfusion in order to restore blood volume. In such cases, extreme caution is indicated and frequent examination of the patient during transfusion to detect overloading of the circulation is imperative.

Prophylaxis.—This depends (a) on the clinical acumen used in the diagnosis of the underlying disease and in the assessment of the patient's cardiovascular status and (b) on the rate of administration of the transfusion.

Treatment.—As soon as the condition is recognized, transfusion should be discontinued and phlebotomy should be performed. Tourniquets may be placed on all four extremities with sufficient pressure to cause venous stasis, but not for longer than 15 minutes.

Differential diagnosis.—This offers no difficulty if the possible development of cardiac failure be kept in mind.

Retinal Hemorrhages

Retinal hemorrhages have been observed to appear during or shortly after transfusions. They are seen most often in patients with blood dyscrasias or capillary damage.

Infections Transmissible by Transfusion

Malaria and syphilis have many times been transmitted by the transfusion of whole blood. A negative serologic reaction in the donor does not necessarily insure against transfusion syphilis. Several cases are on record in which syphilis was transmitted by the blood of a donor who had primary syphilis but whose serologic reaction had not yet become positive. However, recent work (44) has shown that the Treponema pallidum will not survive over 96 hours in preserved blood. Utilization of blood more than 96 hours old should therefore eliminate transfusion syphilis. The viability of the malarial parasite in preserved whole blood (25) is somewhat longer, but the figures of different observers vary considerably. It has recently been established that infectious jaundice has been transmitted by transfusion of both blood and plasma.

Toxicity of Potassium

When the fact became known that potassium diffused into the plasma from the erythrocytes during storage, it was feared that the high potassium content might prove toxic in tranfusion (40). No evidence of such poisoning by potassium has ever been observed (16).

Transfusion of Cold Solutions

It was formerly considered necessary to warm blood or other fluids before intravenous administration. This practice not only resulted

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in the loss of valuable time and required the use of special equipment, but it was potentially dangerous in the case of blood transfusion. At least one fatality has been reported (5) from the injudicious heating of blood during a transfusion. It has been shown that fluids may be injected intravenously at relatively low temperatures (15° C.) without reaction (17). This means that blood or plasma may be taken from the refrigerator at 2° to 5° C. and used at once, since a temperature of about 15° C. (60° F.) will be attained by the time the solution reaches the recipient's vein after passing through the intravenous set. This fact is exceedingly useful in the transfusion of preserved blood or plasma in emergencies, since it allows all possible haste.

Incidence of Transfusion Reactions

It is apparently true that within the safe period of storage for any preservative mixture described in this manual the incidence of reactions can be held to a low level. If that period of storage is exceeded, spontaneous hemolysis becomes excessive, and the reaction rate will increase. The incidence of reactions seems quite comparable in clinics where proper precautions are taken. Diggs and Keith (51) reported an incidence of 6.7 percent for stored citrated blood. Rosenthal et al. (53) had an incidence of 13.4 percent for citrated blood, fresh and stored. If transfusions of blood stored for over 10 days are excluded, their rate was 7 to 8 percent. Muether and Andrews, (52) using a dextrose-citrate mixture, had an incidence of 5.3 percent for bloods stored up to 90 days, mostly under 30 days. DeGowin and Hardin (20), in a series of 2,423 transfusions of citrated blood (10-day limit) and dextrose-citrate blood (30-day limit), could find no significant difference between reactions in the two preservative mixtures, either fresh or stored. The incidence of reactions in the entire series was 4.8 percent. Alsever (54) in a series of 1,500 transfusions of fresh and stored dextrose-citrate blood (21-day limit) had a reaction rate of approximately 5 percent. No difference was observed in the use of fresh and stored blood.

Chapter V. *Changes in Blood During Storage*

Red Cells

Spontaneous hemolysis begins in minute degree as soon as blood is collected. When it is stored at 2° to 5° C. hemolysis proceeds at rates depending upon the preservative mixture employed (13, 14, 36, 37).

If citrated blood be taken as a standard of reference, the addition of electrolytes, such as sodium chloride, accelerates hemolysis. The addition of dextrose definitely inhibits the rate of destruction of the red cells. The mode of action of the dextrose is not known, but it has been demonstrated that to secure optimum effect, a concentration of about 3 percent dextrose must be attained in the blood mixture. With such conditions, there is less than one-half as much hemolysis in 30 days of storage as occurs in citrated blood at 10 days. Isotonic concentrations of solutions should be used in the preservative mixtures. When appreciably hypertonic solutions are employed, the contents of the erythrocytes become hypertonic during storage so that they may be ruptured when they come in contact with the plasma of the recipient. There is slightly less spontaneous hemolysis when blood is stored in a container from which all the air is displaced by the blood mixture. It has been repeatedly demonstrated (20) that human blood withstands very well the agitation incident to transportation over great distances if the containers are full. Red cells stored in dextrose-citrate mixtures persist longer in the circulation of the recipient than do erythrocytes stored in citrate alone (35).

Other Constituents

The leukocytes lose viability rapidly during storage, very few surviving the fourth day (8). This seems to be independent of the preservative employed. The platelets are even more evanescent (6), disintegrating within a few hours.

The plasma proteins are relatively stable (41) and are not denatured to any significant extent. The prothrombin of the plasma disintegrates slowly during storage at refrigerator temperatures, being about 70 percent of normal at 21 days and about 30 percent in 30 days (32, 46, 50). It should be emphasized, however, that the prothrombin is only one factor in the clotting mechanism and that blood 1 month old will clot promptly when recalcified.

Normally, human red cells contain about 20 times as much potassium as does plasma, while the latter contains sodium to the exclusion of the cells. During storage the red cells lose much of their potassium to the plasma, and sodium diffuses into the cells. This process attains its maximum in about 15 days (15), producing a plasma with a relatively high concentration of potassium.

Some study of the rate of loss of complement and antibacterial substances has been made, and the reader is referred to the original articles for details (28, 29).

B. TECHNIQUE

Chapter VI. *Operation of a Blood Bank*

Advantages of a Blood Bank

The advantages of a blood bank are:

1. The instant availability of blood or plasma in emergencies.
2. The privilege extended to donors of trading their blood for that of the proper type in the bank.
3. Economy to hospital and patients.

Each bank may be modified to suit local conditions, since an organization which is admirable for one set of circumstances would not fit another. An attempt will be made to outline the principal variations and to indicate some of the advantages of each.

Types of Blood Banks

Complete blood and plasma bank.—This type of organization is suitable for a hospital having over 25 to 30 transfusions per week. Donors of all types are accepted as the laws of chance present them. The bloods are kept as whole blood until the limit of storage is attained (determined by the preservative mixture used). If the whole blood reaches the outdating period without being called for, the supernatant plasma is then recovered by aspiration from the cell layer, and the red cells are discarded. This citrated plasma should preferably be stored at room temperature in the liquid state or at minus 15° to 20° C. in the frozen state. Proof of sterility is required before it may be administered. If the transfusion service is large, the turn-over of bloods will be relatively rapid, and there will be few which are ultimately converted into plasma, unless an excess is collected. Where the service is small, the amount of plasma accumulated will be correspondingly increased.

Blood banks with limited blood types.—It is the usual experience that most of the out-dated bloods in a bank belong to the rarer types B (14 percent of population) and AB (4 percent). Since the incidence of group A in the population is about 38 percent and that of group O is 44 percent, a blood bank accepting only those two groups could use 82 percent of the donors presenting themselves. Group O bloods could be given to the recipients of all groups, and group A bloods could be given to groups A and AB. Such an arrangement would merely require the determination of the donor's group before the blood is drawn. This type of a bank will operate successfully for hospitals with smaller transfusion requirements than 25 to 30 per week.

Bank employing group O blood only.—A satisfactory blood bank may be operated for small hospitals by the use of only group O blood.

If these are treated with the group-specific A and B substances (see page 3), they become truly "universal donor" bloods and can be used more safely than untreated group O blood, at least theoretically. This is probably the most satisfactory type of blood bank for the very small hospital.

Plasma bank.—When no attempt is made to store and use whole blood because the transfusion service is too small to make it feasible, blood may be collected at irregular intervals, regardless of type, and the plasma separated from the cells either by sedimentation or centrifugation. The plasma is kept primarily for the emergency treatment of traumatic shock, the treatment of the dehydration and shock accompanying severe burns, and the treatment of other conditions as previously discussed. In the treatment of shock due to hemorrhage, the plasma is usually employed as an emergency measure until a suitable blood donor can be found. There is no limit on the smallness of the plasma bank, but the overhead cost increases and could become prohibitive for each unit of plasma processed.

Methods of Accounting

Several methods have been devised by which the accounts of the bank may be kept. In a large hospital where the medical specialties are organized into services, it may be convenient to carry an account in the bank for each department. When a donor is sent in from a particular service, the account carries a credit of blood in cubic centimeters. When a patient on that service receives a transfusion from the bank, the account is debited by the amount of blood given. This system possesses the convenience of placing responsibility on one house officer on each service, who in turn guards the interest of his service by supervising the interns in the procurement of donors. Although donations are requested on the basis of transfusions given to patients in whom the donors are interested, a surplus of blood frequently accumulates in the bank. With service accounts, the occasional patients requiring transfusion but unable to procure donors may be served from the departmental surplus. This is extremely hard to manage under any other type of accounting system.

In a smaller hospital it may be desirable to have each intern carry an account in the bank. When he procures a donor, he receives credit for the volume of blood, which he may then transfuse into one of his patients. Or the individual physician or the individual patient may have to be credited and debited with blood as the local conditions suggest. Many variations of the foregoing scheme are possible.

Acquisition of "Capital"

After the blood bank has been established, the bloods are collected and dispensed on some definite trading arrangement, but it is necessary

to have a stock of bloods with which to initiate operations. The most practical method for a community hospital is for the members of service clubs, lodges, or churches to contribute blood with which to begin the bank. The practice has been widely adopted and has proved popular. It can be explained that it is a community enterprise and that the mere existence of such a bank is a form of protection for the members of the community.

Personnel

It is essential that the blood bank be under the direction of a laboratory worker who is thoroughly familiar with all the procedures in the typing and cross-matching of blood and is capable of carrying out aseptic technique in the collection of blood and in the processing of plasma. The amount of the technician's time consumed by the operation of a blood and plasma bank will, of course, depend upon the magnitude of operations. It is excellent policy to restrict to a minimum the number of persons responsible for handling the blood. The cross-matching and typing should be done only by experienced laboratory technicians. Too frequently, these important procedures have not been well handled when made the responsibility of interns.

Source of Pyrogen-Free Fluids

Before attempting to establish a blood or plasma bank, one should be satisfied that there is a source of pyrogen-free fluids available in the hospital with which to prepare equipment for intravenous use. The lack of this precaution will tend to discredit operation of the entire bank, since it is the common inclination to ascribe pyrogenic reactions to the blood and plasma rather than to improperly prepared equipment. Most small hospitals very properly employ commercially prepared parenteral fluids which are pyrogen-free. The commercial firms unfortunately cannot control the preparation of the equipment used to give their solutions, and this is frequently the source of pyrogenic reactions. A thorough understanding of the preparation of the equipment and centralization of its preparation in one place is the *sine qua non* of a proper transfusion service. The reader is referred to pages 69–72 of the manual for a description of the proper methods to insure maximum freedom from pyrogenic reactions.

Equipment

The most expensive item required for the operation of the blood bank is a mechanical refrigerator. For small banks a standard domestic model electric or gas refrigerator is satisfactory. Most models have thermostats which are adequate to control the temperature between 2° and 5° C. A continuously recording thermometer is advisable. If a stock of whole blood is to be kept on hand, consideration should be given to substitute arrangements while the refrigerator is

being periodically defrosted. (For detailed description of refrigeration see p. 76.) A centrifuge is necessary for the cross-matching and typing of blood; this may be a relatively small, electrically driven, angle-type machine. If it is desired to separate plasma from erythrocytes by centrifugation in the processing of the plasma, a larger, more expensive type of centrifuge with the proper accessories is required. A laboratory microscope will be used in the compatibility tests. Microscope slides, serologic type of test tubes, medicine droppers, and other laboratory accessories will be required. Facilities for steam and dry-heat sterilization will, of course, be necessary. There is a great variety of transfusion apparatus available, and standard acceptable equipment should be employed. The advantages are great of equipment designed to collect blood and process plasma in a completely "closed system" of the type provided by commercially available vacuum bottles. Completely closed systems are difficult to improvise from ordinary laboratory equipment. If the plasma is to be kept in the frozen state, a small commercial freezing unit maintaining temperatures below minus 15° C. will be required. These are ordinarily employed for the storage of ice cream in retail establishments.

It is desirable to have a suite of at least two adjoining rooms in which to conduct the procedures of the blood bank. One room should be reserved for the collection of blood from donors. This can be divided into several cubicles, each containing an examining table and a small side table. The examining tables should be fitted with arm rests. The side tables should be supplied with iodine, acetone-alcohol mixture, sterile gauze, adhesive tape, and tourniquets. A sphygmomanometer makes an excellent tourniquet. The other room should be fitted for laboratory procedures and the keeping of records; the refrigerator may be conveniently placed there. If the donors must wait their turns, it is desirable to have a waiting room from which the operations of the blood bank cannot be observed.

Chapter VII. *Typing and Cross-Matching of Blood*

There is no laboratory procedure in which the results of erroneous technique or interpretation are more disastrous than in the typing and cross-matching of blood. The direct result of a mistake may be fatal. For this reason it is extremely desirable that these procedures be in the hands of an experienced individual. Most physicians have not troubled to obtain the information nor the experience needed to conduct these tests properly. The printed directions for carrying out these procedures are deceptively simple and give a false sense of security.

Determination of Blood Groups

1. *Equipment.*—The only reagents required for blood grouping are specific and potent agglutinating sera. With satisfactory sera, fairly complete agglutination should be visible to the naked eye within 15 to 20 seconds. The activity of grouping sera should be checked at weekly intervals against known A and B cells in order to avoid the use of deteriorated sera which may become too weak to group all bloods properly.

Equipment required:

a. Glass slides.

b. Wooden applicators or tooth picks.

c. Wax pencils.

d. Capillary pipettes fitted with rubber bulbs or hypodermic syringes fitted with 24-gage needles.

e. Small test tubes, such as 8 by 75 mm.

f. Four percent sodium citrate (dihydric).

g. Physiological saline solution (0.85 percent sodium chloride).

h. Microscope.

i. Centrifuge.

2. *Choice of methods.*—Blood grouping and cross-matching can be carried out by a slide method or a centrifuge test tube method. It is desirable when possible to use the centrifuge test tube method. The slide method is described in detail for blood groupings, the centrifuge method for cross-matching, in order to present both in a minimum of space.

3. *The slide method.*—The test is made as follows:

a. Divide a slide equally with a wax pencil.

b. Place the subject's initials or number in the lower right-hand corner of the slide, the letter "A" in the upper left, and the letter "B" in the upper right-hand corner.

c. The blood should be collected preferably by venipuncture (5 cc.). If this is not feasible, 0.5 to 1.0 cc. may be obtained by deep puncture of a finger or ear-lobe, after cleansing the site with alcohol and allowing it to dry. The blood is placed in tubes containing citrate solution in approximately the proportion $\frac{1}{5}$ to $\frac{1}{10}$ of the volume of blood drawn.

d. A red cell suspension is prepared by mixing one drop of the citrated blood with about 1 cc. of saline solution. With normal blood this gives a cell suspension of approximately 2 percent. In the case of anemic patients, more blood should be added, to make a suspension matching in color that of the donor's. Mark the tubes containing the recipient's blood RC (recipient's cells), and that containing the donor's blood DC. Save the remainder of the blood collected for cross-matching.

e. Place one drop of cell suspension on each half of the marked glass slide.

f. Place a drop of A (anti-B) serum on the left side of the slide and drop of B (anti-A) serum on the right side of the slide.

g. Mix well with a wooden applicator or toothpick (separate end for each side), rock the slide manually 5 to 10 seconds to insure thorough mixing, then allow to stand for 5 to 10 minutes, tilting a few times, about once every minute.

h. In warm climates where the slide preparation is apt to dry up, it should be kept in a moist chamber during the period of observation. (A moist chamber can be made by placing pledgets of wet cotton under petri dishes or glass trays. If the use of a moist chamber is impracticable, the addition of a drop of saline solution to each side of the slide after about 5 to 10 minutes' standing will usually prevent drying.)

i. The reactions are read with the naked eye and under the low power of the microscope, if one is available. In a positive reaction the cells are stuck together in clumps usually visible to the naked eye. The interpretation of the grouping tests is shown below:

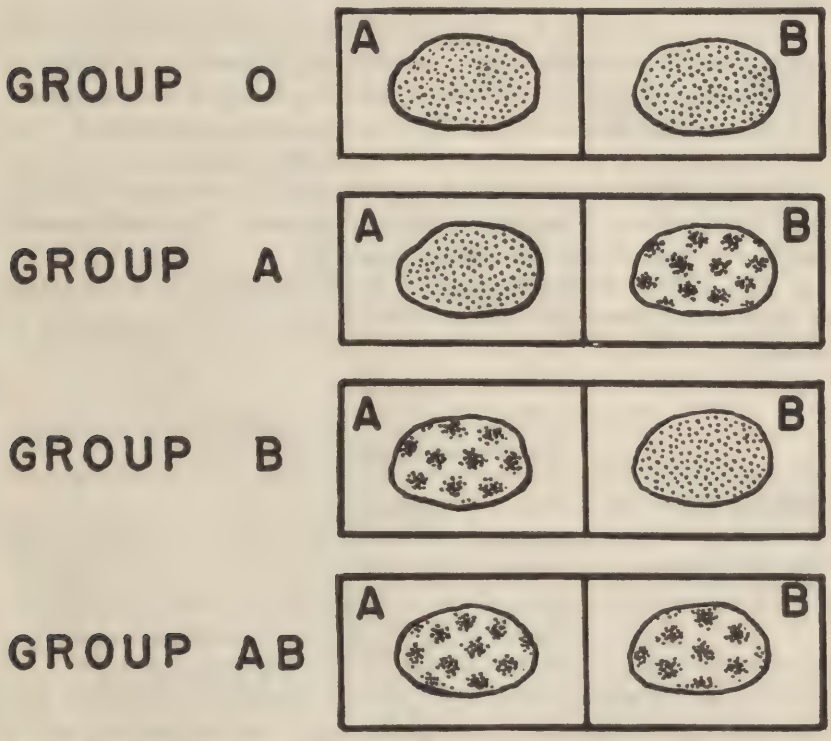

FIGURE 1.—Blood grouping. Fine dots represent no clumping (negative reaction). Massed dots represent agglutination (positive reaction). (After Army Technical Manual.)

4. *Centrifuge method.*—a. Prepare two small test tubes, one marked with the letter "A" and the subject's initials, the other with the letter "B" and the subject's initials.

b. Blood is collected and the red cell suspension prepared as previously described.

c. Place one drop of cell suspension in each tube.

d. Place a drop of A (anti-B) serum in the tube marked "A," and a drop of B (anti-A) serum in the tube marked "B."

e. The tubes are now centrifuged and the reactions read as described in paragraph c, under centrifuge method, page 22.

5. *Confirmation of grouping by the testing of plasma.*—When time permits, and preferably as a routine, the following confirmatory test on the plasma of the individual being grouped should be carried out. This is essential for the certification of universal donors.

a. Divide a slide in halves with a wax pencil and mark the left side "AC" (group A cells) and the right side "BC" (group B cells).

b. On the left half of the slide place a drop of fresh known group A cell suspension; on the right half place a drop of known group B cell suspension.

c. Add two drops of the subject's plasma to each side of the slide and mix each by stirring with a separate applicator or toothpick.

d. Observe the slide for at least 20 minutes, tilting it back and forth at 2- or 3-minute intervals, and then examine for agglutination.

e. This test may also be done by the centrifuge method.

f. If it is difficult to distinguish between true agglutination and rouleaux formation, stir again with an applicator. This will usually break up rouleaux into a uniform suspension.

g. The scheme of identification of blood groups from the reaction of unknown plasma and known cells is given in the right half of table 4.

Table 4

Identification of blood groups

Grouping of unknown cells with known sera		Agglutinogens in cells	Grouping of unknown plasma with known cells		Agglutinins in plasma	Group
A	B		A	B		
−	−	None	+	+	Anti-A and anti-B	0
−	+	A	−	+	Anti-B	A
+	−	B	+	−	Anti-A	B
+	+	A and B	−	−	None	AB

6. *Mass grouping.*—When large numbers of individuals are to be grouped in a relatively short space of time (500 or more per day), certain modifications of the technique just described are necessary.

a. For the sake of expediency the test should be done on glass slides.

b. The slides should have the left and right halves marked "A" and "B" in advance, as indicated in the description of the slide method.

c. A team of three persons should work simultaneously at a table. The personnel to be grouped file past one by one. Accurate blood grouping can be done at the rate of 60 to 90 per hour. The three members of the grouping team may be designated as X, Y, and Z.

d. Member X cleanses and punctures the finger as described. He places a small drop of whole blood, the size of a pinhead, on each side of one of the slides by touching the slide to the drop as it forms. The use of too large a drop may obscure and delay the agglutination reaction. He numbers the slides serially with a wax pencil. To the left side he adds one drop of A serum (anti–B), and to the right side, one drop of B serum (anti–A). He mixes each with a toothpick or applicator, rocks the slide for 10 to 20 seconds, and makes a preliminary reading which is recorded by Z.

e. The individual being grouped has in the meantime passed to member Y, who independently repeats the test, but uses grouping sera *from different bottles.* He also makes a preliminary reading, which is likewise recorded by Z.

f. Both X and Y pass their slides to Z, who rocks them a few times, about once every 5 minutes, and retains each slide until 30 minutes have elapsed. This is important in order to insure that weak subgroups of A, particularly of AB, do not escape detection.

g. In case of discrepancy between the results of the tests carried out by X and Y, or between the readings and the group determined for the individual previously, if any, he should be recalled for regrouping later, when enough blood will be taken for plasma. Then both cells and plasma should be tested.

h. Member Z discards the old slides for washing after the lapse of 30 minutes, at the same rate that new ones accumulate. He keeps records in a book ruled with seven columns: individual's name and number, previous grouping (if any), preliminary reading by member X, final reading of X's slide by Z, preliminary reading by Y, final reading of Y's slide by Z, and final grouping.

Note.—The method described above is reliable only if (1) the sera are of high potency, (2) the size of the drop of blood is small, about the size of a pinhead, and (3) the grouping sera are added before the blood has a chance to dry.

Cross-Matching

After a donor belonging to the same blood group as the patient has been selected, the cross-matching test must be performed before the transfusion is given. In rare instances the bloods of donor and recipient, even though of the same group, are not compatible; that is, there will be some agglutination or hemolysis of the donor's cells by the patient's serum or plasma or of the patient's cells by the donor's serum or plasma. (See pp. 24–25.)

1. *The centrifuge method.*—The test is performed as follows:

a. For the test, use the citrated blood samples obtained from the donor and recipient.

b. Separate the plasma and cells of both donor and recipient by centrifugation or sedimentation.

c. Prepare two small test tubes, bearing the subject's initials, one marked "DP/RC" and the other "RP/DC." In the first, place with a capillary pipette, or syringe and needle, one drop of the donor's plasma (DP) and one drop of the recipient's cell suspension (RC), using a different pipette (or syringe) for each reagent. (If only a single pipette is available, it should be rinsed twice with saline before taking up another reagent.) In the other tube place one drop of the recipient's plasma (RP) and one drop of the donor's cell suspension (DC). Mix, centrifuge at low speed for exactly 1 minute, observe for hemolysis, and then resuspend by gentle shaking. Even if the cells appear to resuspend to an even suspension, examine a drop on a slide under the low power of the microscope for agglutination. Agglutination or appreciable hemolysis should disqualify the donor.

2. *The slide method.*—If no centrifuge is available, the cross-matching may be done on a slide by the following alternative procedure:

a. Divide a clean slide as for the standard grouping test and mark the left side "DP/RC" and the right side "RP/DC," plus the subject's initials or number.

b. Place one drop of donor's plasma (DP) on the left side and one drop of recipient's plasma (RP) on the right, using different capillary pipettes (or syringes and needles) for each transfer.

c. Mix one drop of recipient's cells (RC) with the donor's plasma (DP) and one drop of donor's cells (DC) with the recipient's plasma (RP), using different capillary pipettes for each transfer.

d. The remainder of the test is done in the same manner as for the standard grouping, except that it is necessary to observe the tests for a longer time. Any agglutination or appreciable hemolysis evident within 20 to 30 minutes should disqualify the donor, and others should be tried until one is found whose blood gives no trace of agglutination or hemolysis. This is especially true when there is any agglutination of the donor's cells by the recipient's plasma. Since the slide cross-

matching test requires long observations, precautions to avoid drying should be observed.

Rh Typing and Cross-matching

The indications for testing to rule out Rh incompatibility are given in italics on page 3. It is preferable to eliminate this danger by typing so that only Rh negative blood is used. If no typing serum is available, Rh incompatibility can be demonstrated by special cross-matching.

1. *Typing.*—The tests are set up in small test tubes, following essentially the procedure described for grouping by the test tube method. Small narrow Kahn tubes of inside diameter 7 to 8 mm. are satisfactory.

a. One drop of a fresh 2 percent blood suspension in saline is mixed with one drop of Rh testing serum in a small test tube which is placed in a water bath or air incubator at 37° C. for 1 hour.

b. The reaction is read by gross inspection of the undisturbed sediment in each tube, noting whether the sediment is smooth and compact or rough and diffuse, and by gross and microscopic inspection of the cell suspension after gentle shaking. The tubes are then centrifuged 1 minute at low speed, after which the sediments are again examined in the same way for gross evidence of agglutination, and the results rechecked by microscopic examination of a drop of the gently resuspended cells on a slide.

c. Control tests should be set up, using suspensions of known Rh negative and Rh positive blood cells. In any laboratory where these tests are done, the personnel should be tested in order to have immediately available blood cells of known Rh type for the control tests and for prospective Rh-negative donors.

2. *Cross-matching.*—a. To demonstrate Rh incompatibility, a cross-match, prepared as directed on page 22, should be incubated at 37° C. for 1 hour.

b. Agglutination (RP/DC) indicates Rh incompatibility.

c. Controls should be set up as described in 1c above.

Selection of Universal Donor

1. Donors belonging to group O are often used as universal donors because their cells are not ordinarily agglutinated by the serum of any of the other three groups. Rarely, however, group O individuals are encountered with such potent isoagglutinins that the dilution of their serum in the patient's circulation may not suffice to prevent a hemolytic reaction. Preferably, those group O individuals should be used as universal donors whose sera have been shown not to have excessively high titer isoagglutinins by actual titration. In an emergency, any donor certified as belonging to group O, as proved by complete tests

on cells and plasma, may be used, provided that the blood is transfused slowly.

2. Test to establish acceptability of a "universal donor."

a. Prepare a 1:50 dilution of donor's plasma by adding 0.1 cc. of plasma to 4.9 cc. of saline solution.

b. Place one drop of the diluted plasma on a slide and add one drop of the patient's cell suspension. Mix well with a wooden applicator or toothpick and observe the mixture for about 10 minutes, tilting the slide about once a minute.

c. If no or only weak agglutination occurs within 10 minutes, the titer of incompatible agglutinins is not excessively high, and this donor may therefore be used. If agglutination visible to the naked eye occurs, the donor should not be used. It is known that group O individuals who have previously received transfusions of pooled plasma occasionally develop a dangerously high isoagglutinin concentration.

The use of a universal donor does not obviate the need for cross-matching tests, although in an emergency these tests may be omitted if proved group O blood is used. This procedure is more apt to be safe if the donor is known to have weak isoagglutinins in his plasma and is Rh negative.

Sources of Error in Typing and Cross-Matching

False negative reactions are most often due to the following:

1. Weak titer of sera.
2. Insufficient time of observation of test.
3. Cell suspensions too heavy (the agglutinins may be absorbed by excess of cells).
4. Low sensitivity of agglutinogens.
   a. In children.
   b. The A agglutinogen in AB blood.
   c. The cells of stored blood.
5. The presence of a powerful hemolysin (cell clumps quickly hemolyzed).

Fresh plasma may, in rare instances, give hemolysis instead of agglutination, especially in warm climates. Hemolysis is more frequently observed when fresh serum is employed. Care should be taken not to read this as a negative reaction.

6. Errors in labeling.
7. Erroneous recombination of cells and serum from the same blood in cross-matching.

False positive reactions are usually due to the following:

1. Rouleaux formation (pseudoagglutination). This can usually be differentiated by microscopic appearance; in case of doubt, dilute with saline; rouleaux will break up, true agglutination will not.

In many patients with rapid sedimentation rate due to severe sepsis or other causes, rouleaux formation may be confused with true agglutination when the patient's plasma is grouped with known cells or cross-matched with the donor's blood cells. Rouleaux formation can often be recognized under the high dry power of the microscope by the appearance of loose clumps of red cells with their flat surfaces in contact so as to resemble stacks of coins. Pseudoagglutination should be suspected whenever unexpected clumping is encountered and is almost certain if the patient's cells suspended in his own plasma show a similar phenomenon. However, rouleaux formation is usually broken up by stirring, a procedure which as a rule intensifies true agglutination. Pseudoagglutination can be prevented by repeating the test with 1:2 or 1:3 dilution of the plasma, a dilution usually insufficient to weaken true agglutination.

2. "Cold agglutination" due to agglutinins which react only in cold; very few react at room temperature and none at 37° C.

3. Contaminated sera.

4. Panagglutination may occur in certain diseases (test cells against group AB serum).

5. Autoagglutination (test cells against serum of same blood).

In most individuals the serum contains a weak agglutinin capable of acting on the individual's own cells and also on all other human bloods. Normally, autoagglutination can be observed only at low temperature. In certain diseases, e. g., hemolytic anemias, trypanosomiasis, virus pneumonia, etc., or in the course of sulfonamide therapy, the reaction may also occur at room temperature. Autoagglutination can be recognized by its reversibility at body temperature and by its nonspecificity; i. e., agglutination occurs with *every* human blood, regardless of the blood group, including the blood of the individual from whom the serum is derived. The phenomenon does not, as a rule, affect the outcome of a blood transfusion and is mainly important as a source of error in blood grouping. When the recipient's plasma agglutinates cells of prospective donors of the same group:

1. Rule out rouleaux formation.

2. Prove autoagglutination by testing the recipient's plasma against his own cells.

3. Carry out the cross-matching at 37° C., since the phenomenon does not usually occur at body temperature.

4. If agglutination is still present, a new cell suspension should be prepared as follows: Separate the recipient's plasma (or serum) from the cells at 2 to 5° C., since the autoagglutinins are absorbed by the cells at low temperature. Wash the cells at 37° C. and repeat the cross-matching as in 3.

5. If no agglutination occurs under conditions 3 or 4, it may be presumed that the agglutination phenomenon occurring at lower temperatures was due to autoagglutinins.

6. False agglutination of blood from umbilical cord.

Grouping Sera

1. *Preparation.*—a. *General.*—Strong B serum is difficult to obtain because of the scarcity of B donors in general. It is wise, therefore, always to have an up-to-date list of donors with a note as to the strength of the reaction in each case. Choose a person of the desired group, known to have a potent serum, and take the blood by venipunc-

ture. Enough serum can be obtained from 30 to 50 cc. of blood to last over almost any emergency. Serum which must be used immediately or within 1 to 7 days after collection should be inactivated by heating in a water bath at 56° C. for 30 minutes, to avoid hemolysis which may mask agglutination. Reasonable care should be taken to maintain asepsis while drawing the blood and separating the serum, because sterile serum will retain its full strength for a long time.

b. *Collecting the blood.*—It has been found best to collect blood in sterile stoppered centrifuge tubes or bottles. Allow the blood to clot and then shake the containers gently but sufficiently to break up the clot so that the greatest possible yield of serum can be obtained.

c. *Separating serum.*—Centrifuge the containers of blood at 1,500 to 2,000 r. p. m. to separate the serum from the broken clot, or allow to separate in the refrigerator overnight. Decant or pipette off the clear serum into sterile containers; then recentrifuge, if necessary, to get rid of remaining red cells, and decant into sterile containers.

d. *Preservation of serum.*—When possible, it is desirable to add chemical preservatives to the sera, although in emergencies they may be kept for some time without preservatives, especially if kept cold. The addition of the dyes and the preservative solution to the sera serves both to minimize bacterial growth and to facilitate their identification. If the dyes and mercurial preservatives are not available, the sera may be preserved by adding tricresol to give a final concentration of 0.5 percent.

(1) *Preserving and coloring group A serum.*—(a) Have ready a 1 percent aqueous solution of neutral acriflavin and a 1 percent aqueous solution of phenyl mercuric nitrate, phenyl mercuric borate, or merthiolate.

(b) For each cubic centimeter of clear A serum add 0.015 cc. of the acriflavin solution and 0.01 cc. of the preservative solution.

(c) Mix thoroughly. Store in 2 or 5 cc. sterile vials sealed with rubber stoppers. Keep in refrigerator when not in use.

(2) *Preserving and coloring group B serum.*—(a) Have ready a 1 percent aqueous solution of brilliant green.

(b) For each cubic centimeter of clear B serum add 0.01 cc. of the brilliant green solution and 0.01 cc. of the preservative solution.

(c) Mix and store as described for the A serum.

If long preservation is desired, store the sera in the frozen state in small quantities, e. g., 1 to 5 cc. The frozen sera are then thawed as needed.

2. *Criteria for the selection of potent grouping sera.*—a. *General.*—The criteria for selection of potent grouping sera depend upon biological reactions and are consequently subject to considerable variation.

Several methods are employed in various laboratories for selecting grouping sera. All of these methods are subject to:

(1) Variations in sensitivity of test cells.

(2) Probable variations in properties of the agglutinins in the serum.

(3) Protein concentration employed.

(4) Intrinsic stability of the preparations.

(5) Probably other factors still unknown.

b. The following technique for testing is recommended as one satisfactory method for the selection of potent grouping sera.

(1) *Group A serum (anti-B).*—(a) Minimal titer. Prepare a 1:16 dilution of the serum by mixing 0.1 cc. of serum with 1.5 cc. of saline. Mix one drop (0.05 cc.) of the diluted serum on a slide with a drop (0.05 cc.) of a group B fresh cell suspension, prepared as directed on page 18. If possible, set up a parallel test with a cell suspension from a second individual of group B. Mix with an applicator or toothpick; agitate by rocking the slide to and fro at intervals of 1 minute. The titer of the serum is satisfactory if agglutination readily visible to the naked eye appears in less than 10 minutes with both bloods.

(b) Speed and intensity of agglutination (avidity). Set up a test similar to that described in (a), using *undiluted* serum, and rock the slide continuously. Agglutination must be visible to the naked eye within 15 seconds, and should be complete within 30 seconds.

(c) Specificity. It is recommended that the serum be used to test at least 50 bloods taken at random, in parallel with a known serum, with satisfactory results, before being considered acceptable.

2. *Group B serum (anti-A).*—The tests are performed much as for A, except that account must be taken of weakly reacting A agglutinogens (A_2 and A_2B).

(a) Minimal titer. Test as described for A serum. The 1:16 dilution should agglutinate A_2 cells within 10 minutes.

(b) Speed and intensity of reaction (avidity). Test as described for A serum against two suspensions of cells, at least one of them of subgroup A_2. Distinct clumping should be visible within 60 seconds with the A_2 cells and within 15 seconds with the A_1 cells.

(c) Specificity. Carry out tests similar to the foregoing with 50 bloods taken at random, and include if possible at least one blood of subgroup A_2B.

3. *Identification of A_1, A_2, and A_2B bloods.*—The simplest method is to use a commercial absorbed B serum (anti-A_1), if obtainable. This agglutinates bloods of subgroups A_1 and A_1B, but not those of subgroups A_2 and A_2B. The next best procedure is to test a series of known A and AB bloods with one or two weak group B sera. Usually

some of the bloods are definitely more weakly agglutinated than others. These weakly reacting cells are used as A_2 (or A_2B) in the foregoing tests.

4. *Anti-Rh testing sera.*—Potent sera may be obtained from the blood of mothers who have borne babies with erythroblastosis fetalis. However, only about 1 in 50 of these mothers yield serum of a titer high enough to be satisfactory for testing purposes. Before distribution for use, the testing serum should have had its anti-A and anti-B agglutinins (if present) neutralized by the addition of group-specific substances.[1]

Chapter VIII. *Collection of Blood*

Psychology of the Inexperienced Donor

The inexperienced blood donor must be treated with due regard for his psychological state when he presents himself at the blood bank. The immense amount of publicity released in the last 5 years has already acquainted the average layman with the facts of the four blood groups and the question of incompatibility of bloods in transfusion. He also knows something about the operation of blood banks. If he has never actually given blood, however, he may have certain apprehensions which can easily be enhanced to the point where untoward symptoms may develop if he is made to wait too long before bleeding is actually performed, or if he is carelessly allowed a glimpse of a flask of blood or an intimate view of the blood-letting of other donors. Tact in meeting the prospective donor and promptness in dealing with him will prevent considerable distress to him and embarrassing delay or failure for the operator.

Donor's Release

It is a moot question whether to require the donor to sign a release stating that he had been fully informed as to the use to be made of his blood and that he accedes to the arrangement. Legal opinion is not agreed as to the value of such a document or as to its necessity. Many hospital administrators prefer not to request a written agreement, believing that this only accentuates the importance of the procedure in the eyes of the donor. However, it is the usual practice at present to require a release.

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[1] Anti-Rh testing serum is now being prepared in most of the large medical centers. Limited supplies are now obtainable from Dr. Louis K. Diamond, Children's Hospital, Boston, Mass.; Dr. Philip Levine, Newark Beth Israel Hospital, Newark, N. J.; and Dr. Alexander S. Wiener, Jewish Hospital, Brooklyn, N. Y.

A reasonably satisfactory form of release is as follows:

"I, the donor described herein, voluntarily donate my blood to _____ Hospital, to be used as determined by said hospital. I am giving this blood at my own risk, and agree that neither the hospital nor any members of its staff shall be in any way responsible to me or my heirs for any consequences resulting to me from this procedure."

Many institutions use only a statement similar to that in first sentence suggested here.

Selection of Donors

Donors must be selected by means of the standards (age, history, physical examination, and laboratory tests) described in part I, chapter II, The Donor, page 4.

The Use of Fasting Donors

The use of fasting donors is not absolutely essential, but is desirable. Blood from nonfasting donors may produce a "milky" or fatty plasma. Such plasma produces no untoward reaction in the recipient except an occasional instance of urticaria.

It is recommended that donors have no fatty food for 12 hours prior to bleeding, but they may have any desired quantity of carbohydrate and protein food. A minimum period of 4 hours of fasting should be observed in any event.

It is desirable to bleed donors during the forenoon if possible. The breakfast should consist of fruit juices, dry toast, coffee without cream, fruits, etc.

Preparation of the Donor

1. Place the donor in the recumbent position while the blood is being collected.
2. Determine the arm vein most suitable for venipuncture.
3. Bare the arm to the shoulder. A blood pressure cuff, folded to one-half its width, is applied to serve as a tourniquet. It is advisable to apply the cuff in the reverse position so that the tubing will be away from the site of venipuncture.
4. Prepare the skin with acetone-alcohol mixture [2] and follow by 7 percent iodine. Remove the iodine with acetone-alcohol mixture.
5. Saturate a sterile gauze pad with the acetone-alcohol mixture and apply it over the area selected for venipuncture.
6. Raise the pressure in the cuff to 40–60 mm. of mercury.
7. Using a small syringe and a 26- or 27-gage needle, raise a small intradermal wheal at the site of venipuncture with a suitable local anesthetic, such as 1 percent solution of procaine. After making the injection, replace the gauze pad saturated with acetone-alcohol mixture

[2] Acetone-alcohol mixture: 10 cc. of acetone and 90 cc. of 70 percent alcohol.

over the wheal and prepare the blood bottle and donor set. If there is a delay in making the venipuncture, the pressure in the blood pressure cuff should be released, as prolonged pressure of the cuff produces pain and has a poor psychological effect on the donor, thereby increasing the tendency to fainting. (See first paragraph, ch. III, p. 47.)

Care of the Donor During Bleeding

1. If the donor pales and the flow of blood decreases, give aromatic spirits of ammonia, drams 2 in water, by mouth.

2. Waiting donors should be kept out of the bleeding room until their turn and should be segregated from the donors who have preceded them.

The Technique of Collection

If a commercial vacuum apparatus is employed, the closure of the evacuated flask containing the anticoagulant is uncovered aseptically, and the needle of the special donor valve is inserted through the closure, the valve being completely closed. A 15-, 16-, or 17-gage needle is then inserted in the donor's vein; as soon as it is evident that this has been accomplished, the donor valve is opened gradually allowing the vacuum in the flask to give the proper rate of flow of blood. The flask is preferably held in an inverted position and is gently moved so that the contents are set in motion, thoroughly mixing the blood with the preservative mixture. In the meantime, the donor has been instructed to open and close his fist slowly and repeatedly to aid the flow of blood. When sufficient blood has been collected, the donor valve is closed, the tourniquet is released, the needle is withdrawn from the donor's vein while a gauze sponge is pressed over the site of the puncture, and the donor is requested to open the hand and elevate the arm. A pressure bandage is placed on the antecubital fossa for a few hours. (See also pp. 47–49, 56–58.)

The donor valve is now withdrawn from the closure of the flask and the blood in the rubber tubing is utilized as follows: Two or three drops are allowed to flow into a small test tube containing 3 to 5 cc. of the preservative mixture, and the remainder is drained into a serology tube. Before the latter is used for the serologic tests, two or three capillary pipettes (drawn from glass tubing) are filled with serum, sealed, and placed in the tube containing the cell suspension. This is now clearly marked and attached to the flask of blood as the "pilot tube," to be used later for typing and cross-matching. The flask of blood mixture should be appropriately labeled, thoroughly mixed by repeated inversion of the bottle for 1 minute, and promptly placed in the refrigerator.

It is possible to collect blood satisfactorily in a commercial vacuum flask without the use of a donor valve. This may be accomplished

in two ways. In one method a length of heavy-walled rubber tubing is employed, in both ends of which are inserted 17-gage needles. One needle is inserted into the donor's vein and, as soon as the blood is flowing into the tubing, the other needle is thrust aseptically through the stopper of the vacuum bottle. The gage of the needles is such as to properly control the rate of flow. The collection of blood is discontinued by first withdrawing the needle from the flask. In the other method, the flow of blood is controlled with a screw clamp. Therefore, larger gage needles may be used if desired, since the clamp serves the same purpose as the donor valve.

When the preservative mixture "4" is used (see p. 33), the collecting flask and contents should be ice-cold before the blood is drawn into it (14). This procedure is extremely important to obviate extensive hemolysis from the diffusion of dextrose into erythrocytes. If outside temperatures are high, the procedure is also advised when blood is collected in any preservative mixture.

Care of the Donor Following Bleeding

1. The donor should be allowed to rest in the recumbent position for 15 to 30 minutes.

2. It is advisable that a physician examine the donors before they are permitted to leave the bleeding room.

3. The donor has given blood voluntarily and should be shown every possible consideration. It has been found that a high percentage of donors volunteer their blood again if they have been treated well on their first visit.

4. Coffee and crackers, or similar food, should be given to the donor after the bleeding. This often prevents delayed syncopal reactions. (Orange juice or other sugar-containing drink given before the collection of blood is also very helpful.)

Reactions of the Donor

If the donor is experienced, he may be allowed to leave the room almost immediately. Unpleasant symptoms may be expected in 1 to 5 percent of the inexperienced donors. They may feel well until they attempt to resume the erect position, when extreme pallor develops, sometimes followed by syncope. The pulse is frequently slow. Occasionally, there is nausea and vomiting. A rest of 30 minutes in the horizontal position will prevent these symptoms in most instances. The administration of very hot or very cold drinks is ill advised, because this increases the incidence of reactions. However, it is recommended that some food and fluid be given after blood letting. Donor reactions may also be caused by too rapid a withdrawal of blood (a rate in excess of 100 cc. per minute is not recommended).

A few individuals seem to retain for some hours a tendency to lowered systolic blood pressure when the erect position is assumed. Accidents have occurred when donors have been released after a 30-minute period of observation. There is some danger that if the donor leaves the range of observation too soon he may develop syncope and sustain injuries, such as skull fracture. Two other types of reaction have been noted occasionally in large series of bleedings. The donor may develop transitory, generalized convulsions during the collection of blood. Another rare type of reaction is the occurrence of tetany with carpopedal spasm and a positive Chvostek's sign.

Chapter IX. *Storage of Blood*

The whole blood is stored in the refrigerator with temperatures usually ranging from 2° to 5° C.[3] A little latitude may be allowed in the upper limit of temperature, but reduction below 0° C. will, of course, result in hemolysis. The blood flasks should be shielded from direct sunlight, since most of the rays of the spectrum exert some hemolytic effect.

Within 24 to 48 hours the erythrocytes have settled considerably, and the cell layer is covered by a thin gray layer termed the "buffy coat." This consists of leukocytes, platelets, and debris. Above this is the supernatant layer of plasma. This varies in color and turbidity in different bloods. The presence of lipemia is insignificant, but it imparts to the plasma a uniform cloudiness which is quite characteristic. Increase in turbidity over a period of a few days is an indication for culture. In the course of 5 to 10 days of storage, the supernatant citrated plasma develops a heavy cloud which has been variously called fibrin, fibrinogen, and gamma globulin. This is not noted, or is very slight, in plasma diluted with a dextrose solution. As hemolysis proceeds, a layer of free hemoglobin diffuses upward through the plasma, coloring it red. *The rate of this hemolysis depends on the preservative mixture employed.*

The following preservatives are recommended for specific purposes with their limitations stipulated:

1. *Blood-Citrate*

Composition: 500 cc. of blood.
70 cc. of 2.5 percent or 50 cc. of 4 percent sodium citrate (dihydric) in distilled water.

Recommended purposes:

a. For storage as whole blood for 5 days, during which it may be used for whole blood transfusions.

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[3] See Appendix A, Section II.

b. For conversion to plasma by centrifugation within 48 hours after collection, or by sedimentation for 4 to 7 days.

Comment:

a. Inferior for storage of whole blood, since it does not inhibit hemolysis.

b. There is not sufficient dilution to allow recovery of plasma economically by sedimentation.

2. *Blood-Dextrose-Citrate* (dilution ratio 1 to 0.5) (56)

Composition: 500 cc. of blood.
150 cc. of 5.4 percent dextrose in distilled water.
100 cc. of 3.2 percent sodium citrate (dihydric) in distilled water.

Recommended purposes:

a. For the storage of whole blood suitable for transfusion for 18 days.

b. For the transportation of blood over long distances.

Comment:

a. Minimum added bulk, but a greater tendency to precipitation of fibrin during storage than with solutions 3 and 4.

3. *Blood-Saline-Dextrose-Citrate* (dilution ratio 1 to 1) (4)

Composition: 500 cc. of blood.
500 cc. of solution containing *per liter:*
18.66 gm. dextrose (anhydrous).
4.18 gm. sodium chloride.
8.0 gm. sodium citrate (dihydric).

Recommended purposes:

a. For the storage of whole blood suitable for transfusion for 21 days.

b. For the transportation of whole blood over long distances.

c. For conversion to plasma by aspiration of the supernatant dilute plasma after 16 days of sedimentation or after the whole blood has been outdated.

Comment:

a. The bulk might be considered disadvantageous for some purposes.

b. Optimum plasma recovery.

4. *Blood-Dextrose-Citrate* (dilution ratio 1 to 2.5) (13, 14)

Composition: 500 cc. of blood.
650 cc. of 5.4 percent dextrose in distilled water.
100 cc. of 3.2 percent sodium citrate (dihydric) in distilled water.

Recommended purposes:

a. For the storage of whole blood suitable for transfusion for 30 days.

b. For the transportation of whole blood over long distances.

c. For conversion to plasma of outdated blood by the aspiration of the supernatant dilute plasma.

Comment:

a. The bulk might be considered disadvantageous for some purposes.

b. When blood is collected in this mixture, the flask and preservative must be ice cold, in order to avoid the danger of spontaneous hemolysis.

Solutions 2, 3, and 4 all employ dextrose in an effective final concentration (0.5 to 3 percent.) The duration of adequate preservation in vitro depends upon the dilution employed. Clinical experience indicates that, within the storage limits prescribed for each solution, the life of the stored red cells after transfusion compares very favorably with 5-day-old citrated blood. The available experimental data confirm this. Present evidence would indicate that the best recovery of dilute plasma is obtained from the 1 to 1 dilution (solution 3).

Chapter X. *Transportation of Blood*

It has already been noted that the agitation incident to transportation has little effect on human erythrocytes. However, the blood-preservative mixture should fill the container used, preferably with displacement of practically all the air. This permits only a minimal amount of shaking and an optimal carbon dioxide tension for preservation. When preserved whole blood is contained in flasks with watertight closures, it may be transported most easily by immersing the flasks in ice water. The water-ice mixture will maintain an even temperature as long as unmelted ice is present. The ice may be replenished as often as necessary in the course of the trip. A 10-gallon milk can will accommodate 10 commercial 1-liter flasks and sufficient cracked ice to maintain low temperatures for nearly 24 hours during summer heat, provided an insulated shipping jacket is employed (20). Special precautions are necessary if dry ice (carbon dioxide) is used, since there is danger of freezing the blood. When water-ice mixtures are used, care should be taken not to lower the freezing point by the addition of sodium chloride, sugar, or other substances. Waterproof labels must, of course, be provided if the flasks are to be immersed in water. This type of refrigeration can be used as an emergency measure when the mechanical refrigeration of the hospital fails.

Chapter XI. *Administration of Blood*

When a transfusion is required, the blood group of the recipient is determined and his blood is cross-matched with a blood of suitable

group in the bank. The specimen in the "pilot tube" is used for this purpose.

Equipment

Two items of equipment are needed in the administration of preserved blood: a device for the initiation of the transfusion, and a filter for the blood. The initiation of the injection of blood presents the problem of excluding the donor's blood from the needle until the backflow of the recipient's blood indicates that the needle is in the vein. Several types of procedures are used to accomplish this. The needle may be attached originally to a Luer syringe which is detached after receiving the backflow; the needle is then attached to the line of flow by an adapter. Another method is the use of a Kaufmann side-arm Luer syringe. The backflow is then pulled into the barrel of the syringe up to the side-arm, where the donor blood then enters and reverses the flow. Another procedure is the employment of an accessory line of physiologic saline, which is first run through the system until the successful insertion of the needle in the vein is demonstrated; the donor's blood is then shunted in, either by means of a Y-tube and pinch clamps or by a two-way stopcock. All of the methods mentioned so far are adapted to the administration of blood through a "closed" or nearly "closed" system. When an "open" method is employed, a single line of tubing is attached to a receptacle such as a Kelly bottle or a salvarsan tube. The saline may be poured into the flask and the infusion satisfactorily initiated; then the blood mixture may be added to the infusion.

In the administration of both fresh and preserved blood, a filter should always be employed to remove the particulate matter. Small shreds of fibrin and debris from the leukocytes, which might plug the needle or serve as emboli, are more numerous in preserved blood. When an open system of administration is used, the blood may be easily and adequately filtered through three or four thicknesses of sterile surgical (washed) gauze. Filtration in the closed system offers more difficulties since the limited filtration area frequently becomes completely occluded, and it becomes necessary to use more than one filter. It is hoped that more adequate filters will soon be commercially available.

Rate of Administration

The chief factor governing the rate of administration of the trans fusion is the caliber of needle employed. For the adult recipient, a needle of 18-, 19-, or 20-gage may be used. The blood is usually delivered from a height of about 3 feet above the recipient's arm. With such a system and an 18-gage needle, the maximum volume of delivery is about 25 cc. per minute with citrated blood and about 36 cc. per

minute with blood-dextrose-citrate. The rate may be diminished by constriction of the tubing with a screw clamp. These or faster rates may be advisable in the treatment of shock. The average rate of administration of 10 to 20 cc. (150 to 300 drops) per minute should not ordinarily be exceeded.

Selection of a Vein

The selection of a vein for the insertion of a needle is a matter of experience, and further discussion is probably valueless. It goes without saying that the ability to place a needle in a vein is the *sine qua non* of all transfusion procedures. Occasionally dissection and cannulation of the vessel may be necessary. Attention is directed to the fact that femoral vein injection can usually be performed when all other veins are inaccessible because of severe burns, anasarca, obesity, or extreme shock.

Danger of Warming

As has been mentioned previously, blood, plasma or other fluids may be transfused without preliminary warming. This is necessary when preserved blood is employed, since it not only saves valuable time but excessive hemolysis may be caused by too rapid warming.

Care of Recipient

The recipient should be attended during the transfusion, not only so that the apparatus may be regulated but also in order that the transfusion may be discontinued at the first sign of a severe reaction of the urticarial, the hemolytic, or the circulatory type. It is known that hemolytic transfusion reactions, as a rule, are evidenced by severe symptoms before 75 to 100 cc. of blood have been administered, and, furthermore, that fatal reactions can almost always be avoided if an incompatible transfusion is stopped as soon as symptoms first appear. It is recommended, therefore, that the first hundred cubic centimeters of blood be administered slowly and under the supervision of a physician. It is also recommended that a physician remain in attendance during the entire transfusion in cases where a patient with cardiovascular disease must be transfused. There is great danger of overloading of the vascular system in such patients.

Laboratory Study of the Recipient

Transfusion reactions should be dealt with as indicated in chapter IV. If circumstances permit, it is desirable to examine the urine just prior to the transfusion and ascertain whether it is alkaline or acid in reaction. The result of such a test in the presence of cystitis is, of course, no true indication as to the reaction of the urine as it comes from the kidneys. The urine may be alkalinized by the oral admin-

istration of sodium salts such as bicarbonate or citrate. This may take several hours. Sodium r-lactate may be used intravenously with almost immediate effect. This is thought to minimize the danger from hemolytic transfusion reactions.

In the event of a transfusion reaction the urine voided for 24 hours after the transfusion should be saved, and the volume output should be recorded, since this may be the only indication that oliguria or anuria has developed in a severely ill patient. The urine should also be tested for the presence of hemoglobin.

Chapter XII. *Administration of Red Cell Suspensions*

The preparation of plasma by centrifugation has made available an inexpensive means of obtaining red blood cells for the treatment of anemic patients. In making use of such therapy, one should, of course, be sure that a patient's need will be wholly, or in a large part, met by the transfusion of red cells alone. Reports of this procedure have recently appeared in the literature (57, 58, 59, 60, 61).

1. Citrated whole blood, from which the plasma has been removed, can be used for a red cell transfusion up to 96 hours after collection of the blood, provided the cells are stored at 5° to 10° C.

2. If no plasma is available for a check on the cell typing and for cross-matching, the cells should be typed with two different lots of grouping sera.

3. Unless the red cell residue can be positively identified with the sample in the pilot tube, cells must be removed from the centrifuged residue for typing and subsequent cross-matching with the recipient's serum.

4. Resuspend the cells in 100 to 250 cc. of known pyrogen-free normal salt solution immediately after the plasma has been aspirated. It seems likely that the use of one of the dextrose preservative solutions as the diluent would permit the 96-hour storage limit to be prolonged.

a. If commercial vacuum-type containers are used, the integrity of the closure of the bottle is usually destroyed when the plasma is aspirated. Therefore, the red cell residue should at once be aspirated into a fresh bottle containing the desired amount of diluent in order to assure continued sterility of the cells. A sample for typing and cross-matching can be obtained from the aspirating set.

b. With reusable equipment, the technique differs. Here the bleeding stopper is removed aseptically after the plasma has been aspirated,

and the desired amount of diluent poured into the bottle. It is then immediately closed aseptically with a sterile solid stopper, after removal of a sample for typing and cross-matching.

5. If appreciable hemolysis or change in the color of the cells occurs before use, the bottle should be discarded. (A violaceous discoloration develops with contamination.)

6. Sterility tests on resuspended cells should be carried out routinely while the technique is being established, and periodically thereafter as a check on the procedure. The risk of contamination is minimized if a closed system is employed. Samples for testing should be taken at the time of resuspension.

7. Like whole blood, red cell suspensions should not be warmed and must be filtered either immediately before or during administration.

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Part II. The Processing and Use of Citrated Human Blood Plasma

Chapter I. *General Considerations*

Experimental and clinical observations have established the therapeutic value of citrated normal human plasma. Plasma has been popularly regarded as a blood substitute. This is improper because not all of the functions of whole blood are possessed by plasma, and, conversely, plasma is successfully employed in some clinical conditions in which the injection of whole blood is neither indicated nor therapeutically suitable. It is proper, therefore, to regard blood and plasma as having different fields of application as shown in table 3, page 6. Detailed discussion of the therapeutic use of plasma in shock may be found in OCD publication 2212, "The Clinical Recognition and Treatment of Shock," and in burns in OCD publication 2203–1, "The Treatment of Burns and Prevention of Wound Infections."

Plasma can be prepared at small expense, may be transported without risk of deterioration, and may be stored for long periods of time. No serious reactions follow its administration even in large and repeated doses, and it is available for immediate administration.

In an appraisal of the value of any therapeutic agent, first consideration must be given to the possibility of its harmful effects. Human plasma, properly prepared from citrated blood collected from healthy donors, can be administered intravenously to patients without regard to blood grouping and without reactions, save for occasional mild urticarial manifestations. To serve its purpose fully, blood plasma should be available in a form requiring a minimum of time for its administration, as well as in a form most nearly meeting the requirements of the circumstances under which it is to be used or transported.

In the development of methods of plasma preparation, it must be remembered that liquid plasma is a good culture medium and that bacterial contamination occurs with comparative ease. In addition, certain unstable plasma proteins have a tendency to flocculate or lose their specificity. Pyrogens and bacterial contaminations are apparently responsible for the febrile reactions which have been reported following the administration of plasma. Contamination can be

greatly reduced by the use of aseptic surgical technique during bleeding and the employment of a closed system throughout the processing procedure. Reactions due to pyrogens may be reduced to a minimum by scrupulous care in the preparation of the fluids and equipment used in the preparation and administration of plasma. If it is desired to preserve the unstable protein fractions, the sterile plasma must be promptly fixed by bringing it to the frozen state within a minimum of time after bleeding.

Essential Requirements for Plasma Production

Irrespective of the agency which undertakes to prepare citrated normal human blood plasma intended for intravenous administration, there are certain essential minimum requirements which must be observed without variation. These requirements include the protection of the donor, the method of drawing and processing the blood, qualifications of the laboratory personnel and its medical responsibility as required by law, and the storage of the finished product. These minimum requirements are itemized as follows:

1. The donor must be in such physical condition that the taking of the desired amount of blood will not endanger his health. (See p. 4.)

2. The donor must be free from any diseases transmissible by blood transfusion, as determined by those methods of examination which are considered adequate by competent authority within the jurisdiction of the processing laboratory. (See p. 4.)

3. The bleeding must be done in an adequately equipped bleeding center which conforms to such municipal, State, or Federal laws as are applicable.

4. The bleeding must be under the immediate supervision of a qualified doctor of medicine, assisted by the necessary trained personnel.

5. The bleeding and all the subsequent steps involving the plasma fraction, until it is injected into the recipient, must be carried out in a closed system. (A closed system is defined as an apparatus which will permit nothing to be drawn into the system at any point except the liquid under transfer and the air required for replacement when negative or positive pressure is applied at the proper place. All air for replacement must first pass through a suitable antibacterial filter.)

6. The blood must not have undergone excessive hemolysis. This can be determined by the color of the plasma following centrifugation. A hemoglobin content of 50 mg. percent[4] is considered to be within safe limits. Directions for preparing a satisfactory color standard for comparison are found in paragraphs 1 and 2 of section II, appendix B, page 78.

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[4] 25 mg. percent is the maximum allowed by the National Institute of Health regulations governing commercial preparation of plasma.

7. All glassware coming in contact with the blood or plasma should be clear glass and of good quality (preferably high quality ampoule glass). All rubber stoppers should be of high grade "sulfur-free," nonoxidizing rubber and suitable for stoppering biological products having a high protein content. Each piece of equipment coming in contact with either the blood or the plasma must have been made scrupulously clean by washing in suitable cleaning solutions followed by adequate rinsing with pyrogen-free distilled water or physiological solution of sodium chloride. All exposed parts must be adequately covered by suitable wrappings or inserted into stoppered test tubes. All equipment coming in contact with either the blood or the plasma must have been sterilized in the autoclave at 121.5° C. (15 pounds pressure) for at least 20 minutes (i. e., each part of the material to be sterilized must attain this temperature for at least the full 20 minutes).

8. The plasma must be sterile, as determined by suitable sterility tests.

9. If the final product is retained in the liquid or the frozen state, it must be placed in a container made of good quality glass, completely sealed by a rubber stopper which will permit the entrance of the necessary needles or trochar for administration to the recipient, and the container must be properly labeled.

10. Explicit instructions should accompany each unit of liquid, frozen, or dried plasma, pointing out the necessity of placing into the lumen of the tube leading from the plasma reservoir to the vein of the recipient a filter adequate for the removal of all particles which are of such size as to be dangerous for intravenous administration. (See "Filter Adequate for the Removal of Particulate Matter," p. 85.)

Chapter II. *Liquid, Frozen, and Dried Plasma*

The degree to which human plasma should be processed depends primarily upon the degree of stabilization of the component parts desired, upon the storage facilities available, upon the amount of handling and transportation involved before the plasma reaches the recipient, and upon the interval likely to elapse before use. For the most frequent usages, namely, the replacement of blood volume in shock, hemorrhage, and burns, the three forms of human plasma (liquid, frozen, and dried) must be considered equal in therapeutic value within the limits of the dating periods allowed. A definite expiration or dating period of not more than 2 years [5] has been recognized

[5] 1 year is the present dating period approved by the National Institute of Health.

for liquid plasma and not more than 5 years for dried plasma. An expiration date for the frozen product has not been officially set, since experience with this form is more limited. However, 3 years is the provisional expiration date, provided the plasma is kept continuously at or below the temperature range permitted. It is anticipated that this time limit will be extended. (See p. 54, par. 7.)

Liquid plasma is the most economical to produce and may be made relatively stable by the addition of dextrose as approved by the U. S. P. XII. It is recommended that sterile 50 percent dextrose solution be added in sufficient quantity to obtain a final dextrose concentration of 5 percent. Assurance must be had that the dextrose is free from pyrogenic substance. Liquid plasma with added dextrose will usually remain free from fibrin clot for the entire dating period, provided it is stored at the prevailing room temperature but not below 13° C. (55° F.). If prepared and stored in the liquid state, it soon loses the unstable protein elements, such as prothrombin and complement, and more gradually any specific antibodies.

Frozen plasma is somewhat less economical to process and requires suitable cold storage facilities for its preservation, which add slightly to its cost. It is superior to liquid plasma in that it has a longer period of usefulness, the freezing fixes the unstable protein elements, and the risk of contamination is greatly reduced. It is desirable to process this with the addition of dextrose so that the fibrinogen will remain stable at room temperature after the plasma has been thawed.

Frozen plasma may be the product of choice in large hospitals, particularly where production needs are large but restricted to processing at infrequent intervals, and also where the demand is irregular. The storage of plasma in the frozen state is particularly advantageous when convalescent plasma is to be used for the treatment of infectious diseases. At the present time it is the product of choice to meet the needs of communities where the exact time of need cannot be predetermined and where this unexpected need may be excessively heavy. This is particularly true in large industrial areas or other places where community-wide catastrophes are apt to occur.

Dried plasma is the most expensive form of plasma to produce because of the time involved, the equipment needed for processing and bottling, and also because of the number and qualifications of the laboratory personnel. It has the advantage of stability under the most unfavorable circumstances and over a very long dating period. It is easily and quickly restored to the liquid state, provided it has been properly prepared and bottled. Contrary to the prevailing opinion among the uninformed, however, the preparation of dried plasma of U. S. P. quality or better is an exacting task which calls not only

for skill on the part of the operator but, even more, the use of drying apparatus capable of accomplishing the task properly. At present, it should be undertaken only by the large laboratory having adequate staff, financial resources, and a distribution area extending over a very large territory, or where other special factors make the preparation of dried plasma advisable.

Chapter III. *Methods of Plasma Preparation*

Several methods of preparing plasma are detailed on the following pages, including the employment of both commercial and reusable equipment. The method or methods chosen by a particular laboratory should depend upon its needs and facilities. The reader will appreciate that adequate substitutions can be made for many of the individual pieces of apparatus to be described and for the individual steps taken without affecting the quality of the finished product.

A. Centrifuge Method

1. EMPLOYMENT OF COMMERCIAL VACUUM-TYPE CONTAINERS

The bottles must be sterile and of the proper size to fit into the standard centrifuge cup. In addition, the bottle used must provide a closed system for the collection of blood, a self-contained vacuum, and a stopper which will maintain an airtight seal after puncture with a 15-gage needle (37). (It is suggested that the incidence of reactions following bleeding will be reduced if the following procedure is adopted: Collect 500 to 600 cc. if the donor weighs more than 150 pounds, 250 to 300 cc. if less than 150.)

Collection of Blood

The collection of blood should be under the direct supervision of a trained physician. (See pp. 29–32 for preparation and care of the donor.)

1. Expose the rubber stopper aseptically. (This bottle should not contain an air tube unless the collection may possibly be used as whole blood.)
2. Apply 7 percent Tr. iodine to the top of the bottle, leaving an alcohol sponge over the stopper until ready to insert the valve needle.
3. Unpack the sterile donor set and *close the donor valve.*
4. Wipe off excess iodine and alcohol from the stopper of the bottle.

5. Remove the protective glass tube from the donor valve needle and insert the needle through the rubber stopper. Rotate the bottle so that all surfaces will be bathed by the citrate solution.

6. Reapply pressure on the blood pressure cuff (40–60 mm. mercury). The pressure should be maintained near the donor's diastolic pressure during the period of collecting the blood.

7. Remove the glass tube covering the donor needle and insert the needle into the vein selected.

8. Open the valve and allow the blood to run into the bottle. The donor should be instructed to open and close his hand slowly to increase the flow of blood. It is preferable to have the bottle in the inverted position so that the blood will be mixed adequately with the anticoagulant. The bottle should be shaken gently and constantly during the collection of the blood and for at least 1 minute after the collection has been completed. This further insures mixture of the blood with the sodium citrate solution. Failure to agitate the bottle properly during the period of collection may result in clotting.

If during the collection period there is a fluttering sound at the donor needle, close the valve for 2 or 3 seconds and then reopen it slowly. The fluttering is caused by attempting to draw the blood faster than the vein can be filled. The flutter valve action is undesirable, as it has a definite tendency to hemolyze the blood. The rate of flow should not exceed 100 cc. per minute. After a little experience the operator can judge the desired rate of flow.

9. If two 300 cc. containers are to be filled with blood, prepare the second bottle as in step 5, then shut off the donor valve and transfer the valve to second bottle. Open the valve and proceed as in step 8.

10. After obtaining the desired amount of blood, *close the valve of the donor set*, release the pressure on the cuff, and remove the donor needle from the vein. The bottle should be below the level of the arm when the needle is removed from the vein; this prevents leakage of blood from the donor needle.

11. Maintain pressure with a sterile gauze pad over the site of venipuncture for at least 5 minutes after withdrawing the needle. The arm should be raised for the first 1 or 2 minutes.

12. Withdraw the needle from the stopper of the bottle.

13. Allow 2 or 3 drops of blood from the donor set to flow into a test tube containing 3 cc. of sodium citrate, 2½ percent, in normal saline (if blood typing is desired).

14. Remove the donor needle from the rubber tubing, open the donor valve and allow the remainder of the blood to run into the serology tube. (See p. 65 for material for cross-matching when collection may be used as whole blood.)

15. The donor set must be cleaned immediately after use. (See instructions for *cleaning and preparing donor set*, p. 71.)

Storage of the Blood

1. Place the blood in an icebox (2° to 5° C.) preferably *within* 1 hour after collection. This temperature must be maintained until the blood is ready for centrifugation. The blood container must not be opened from the time of the bleeding until the final preparation of the plasma. Twelve to twenty-four hours of storage is preferable before centrifugation.

2. *Do not attempt to pool whole blood*, as it produces an undue amount of hemolysis.

3. A preservative (bacteriostatic agent) must not be added to whole blood at any time.

4. Freezing of whole blood must be avoided, *as it produces marked hemolysis.*

Centrifugation of Whole Blood

Any rapidly spinning object must be perfectly balanced in order to rotate freely. One of the most important steps in the preparation of plasma is to have the spinning objects (blood, bottles, and trunnion cups) in perfect balance during the period of centrifugation. If this step is performed in a hasty, careless manner, excessive vibration develops, and there is danger of breaking the bottles and damaging the centrifuge. The trunnion cups and bottles *must be perfectly balanced* for the satisfactory separation of plasma.

1. *Balancing of Cups and Bottles Before Centrifugation.*—A good torsion balance is essential for this step. Each cup should be filled with water up to the shoulder of the bottle. This reduces the danger of breakage.

a. *First method.*—If the cups and bottles are weighed against one another, it is absolutely essential that the torsion balance be perfectly level. After balancing by this method, reverse the position of the cups on the torsion balance. If the cups still balance, they may be placed opposite each other in the centrifuge. If the cups fail to balance when reversed, the torsion balance is not level and must be made so before the balancing proceeds.

b. *Second method.*—Another method of balancing is to place weights on one pan of the torsion balance and then adjust the slide beam weight until the cup and weights balance perfectly. Considerable time can be saved if the operator selects the bottle containing the greatest amount of blood for the first balancing; then the other cups and bottles must be balanced to weigh the same as the master bottle.

c. *Achieving balance.*—This should be accomplished by adding rubber bands of various sizes to the balance tray containing the lighter cup and bottle. After balance has been achieved in this fashion, the rubber bands should be placed around the neck of the bottle. This places the additional weight in the correct location on the bottle, which is essential for high speed centrifuging.

2. *Centrifuging.*—a. After all bottles have been accurately balanced, place them in the centrifuge in the proper location and start the centrifuge slowly. If excessive vibration develops, stop the centrifuge, reweigh the cups and start the centrifuge as before. All centrifuges develop some vibration at the critical speed, 500 to 750 r. p. m.

b. Centrifuge at 2,000 to 2,500 r. p. m. for 1 hour.

c. When centrifuging is complete, turn down the rheostat gradually, and when the speed of the centrifuge approximates 800 r. p. m., turn off the switch and *disengage the brushes.* If the centrifuge is stopped too rapidly, there will be an undue amount of red cells in the plasma, and the cell pack will be greatly disturbed. *Do not use the centrifuge brake at any time.*

d. Remove the bottles from the centrifuge and place in a refrigerator preferably for 6 to 24 hours. The plasma should not be aspirated immediately after spinning if swirling of cells occurs following centrifuging, but should be allowed to stand for at least 6 hours to allow the red cells to settle out completely. If swirling does not occur, it is permissible to aspirate the plasma immediately.

Pooling of Citrated Liquid Plasma

It is the consensus that undiluted liquid plasma should be pooled in order to reduce the titer of the agglutinins present. (A 2,000 cc. flask containing 200 cc. of 50 percent dextrose is the minimum size recommended and will average six to eight bleedings.)[6]

1. Check the laboratory tests and make sure that only serologically negative blood is used.

2. Prepare the blood bottles in the following manner:

Expose the stopper aseptically and apply 7 percent Tr. iodine to its top. (Be sure any indentations are thoroughly cleaned with iodine.) Leave an alcohol sponge on the top of the bottle until ready to start aspirating.

3. Prepare the pooling bottle in the following manner:

a. Remove the protective covering from the top of the bottle.

b. Apply iodine to the stopper. Leave an alcohol sponge on the top of the bottle until ready to insert the valve needle.

4. Unpack the sterile aspirating set. The aspirating needle should be covered by penrose tubing. (See pp. 60 and 72.)

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[6] A minimum of eight bleedings per pool is required by the National Institute of Health.

5. Insert the needle of the airway filter through the rubber stopper of the blood bottle and release the vacuum.

6. Close the valve on the aspirating set and plunge the valve needle through the stopper of the pooling bottle.

7. Insert the aspirating needle through the stopper of the blood bottle and into the supernatant plasma.

8. Open the valve and start aspiration. The aspirating needle must be kept beneath the surface of the plasma to avoid loss of the vacuum in the pooling bottle.

9. A strong beam of artificial light should be focused at the junction of the buffy coat and plasma during the aspiration. This serves as an aid in detecting cellular elements that might be drawn into the aspirating needle. Care must be taken not to aspirate any of the red cells. Do not attempt to aspirate all of the plasma, or a number of red cells will be pulled over into the final container. Aspirate the last 30 or 40 cc. of plasma very slowly to prevent lifting and drawing over the cells. Do not attempt to aspirate the last 10 cc. of plasma. After the plasma from one bottle has been aspirated, leave the aspirating needle in place until the next bottle is prepared for aspiration. *Handle the blood bottles with care to avoid agitation of the red cells.*

10. Continue the aspiration as described until the pooling bottle has been filled or until all plasma has been aspirated.

11. Allow the vacuum to pull over the plasma remaining in the aspirating set before disconnecting the set from the last bottle.

Culturing the Pool

Ordinarily, culturing should not be done until the pool has been allowed to stand for 24 to 48 hours at room temperature. This minimizes the chance of obtaining falsely negative cultures.

1. Aseptically remove the protective covering and apply 7 percent Tr. iodine to the stopper of the pooling bottle.

2. Prepare a sterile glycerinated 50 cc. syringe and attach an 18-gage needle. Aspirate approximately 40 cc. of plasma.

3. Use four tubes of Brewer's medium. Inoculate each tube with 10 cc. of plasma. Each tube (25 by 150 mm.) contains 20 cc. of Brewer's sodium thioglycollate medium. (See p. 62 for additional method.)

4. Additional plasma may be withdrawn for protein determination if desired.

5. Place two culture tubes in an incubator at 37° C. and keep the other two at room temperature (20° to 25° C.).

6. Observe the cultures over a period of 10 days. (For interpretation of positive cultures, see p. 80, par. 16.)

7. If pool and pilot bottle cultures are negative the plasma may be released for use. (See p. 53, par. 10–12.)

8. Keep an accurate, permanent record of all cultures.

Addition of a Bacteriostatic Agent

This procedure, although required of commercial firms by the National Institute of Health, is *optional* in hospital practice. If it is desired to add a bacteriostatic agent, this should be done after aspiration of the sample for culturing.

Merthiolate, or phenyl mercuric borate (or nitrate), has been found to be fairly satisfactory for this purpose. *It must be remembered that these agents are only an additional precaution in the preparation of plasma and will not render contaminated plasma fit for use.* Actually, they are effective only against small numbers of certain types of bacteria. Administration of plasma containing a mercurial preservative, in amounts exceeding 2,000 cc. per 24 hours, may conceivably lead to renal damage.

1. Apply 7 percent Tr. iodine to the stopper.
2. Using a glycerinated sterile syringe, add 1 cc. of a 1 percent aqueous solution of merthiolate per 100 cc. of plasma in the pool bottle. This gives a 1:10,000 final concentration of merthiolate. If phenyl mercuric borate (or nitrate) is used, add 0.6 cc. of a 1 percent aqueous solution per 100 cc. of plasma in the pool bottle. This gives a 1:15,000 final concentration of phenyl mercuric borate (or nitrate).

Filling of Final Containers; Storage as Liquid Plasma

1. At any time after the cultures have been taken, the plasma may be aspirated into the final containers.
2. Clean the stopper of the pool bottle with 7 percent iodine and leave an alcohol sponge over the top of the bottle until ready to start aspiration. (Be sure any indentations are thoroughly cleaned with iodine.)
3. Prepare the final container in the manner indicated in items 1 and 2 under "Collection of Blood." (See p. 47.)
4. Unpack sterile aspirating set and close the donor valve.
5. Puncture the rubber stopper of the final container with the needle of the valve set.
6. Pierce the stopper of the pooling bottle with the air-filter needle to release the vacuum.
7. Insert the aspirating needle through the stopper. The aspirating needle should be inserted well below the surface of the plasma.
8. Open the valve of the donor set and start aspirating into the final container. When the first container is filled, close the donor valve.
9. Prepare the next final container, remove the donor valve needle from the first final container, and insert through the stopper of the next container to be filled. Open the donor valve and start aspira-

tion as outlined in the preceding paragraphs. (The donor valve needle is left in place until the next bottle is prepared, in order to prevent undue exposure and possible contamination of the needle.)

10. After the last bottle has been filled, aspirate the remainder of the plasma in the pool into a final container. This plasma, usually 50–150 cc., should be retained as the pilot bottle for this lot of plasma. (It is desirable to include in this sample 20 to 25 cc. of plasma from the top of the pool bottle.)

11. The pilot bottle is allowed to remain at room temperature for 24 hours and then cultured in the manner indicated under "Culturing the Pool," except that a larger quantity of medium is necessary if a bacteriostatic agent has been added. (See appendix B, sec. IV.) The remainder of the bottles of this lot are labeled and stored in a cool (ordinary room temperature), dark room. The preferable temperature range is 15.5° to 26.6° C. (60° to 80° F.); maximum limits are 13° to 37.8° C. (55° to 100° F.).

12. If the pilot bottle of the lot shows no evidence of contamination at the end of 10 days, this lot of plasma is ready for issue. It is advisable to retain the pilot bottle as a control. That is, it may be retained until all reports have been returned on this particular lot of plasma. As the pilot bottles thus accumulate, they may be pooled, cultured, and issued for use. In addition to its use for the final sterility test, the pilot bottle permits the physician in charge of the plasma unit to have a bottle of plasma from each lot for study at any time desired. (See p. 81, par. 20 and 21; par. 18 does not apply.)

13. Liquid plasma, containing a 5-percent concentration of dextrose, may be safely and satisfactorily stored at room temperature for as long as 2 years.

14. Should liquid plasma prepared by this or other methods be stored in an ordinary refrigerator, this temperature will cause precipitation of large amounts of fibrin, frequently sufficient to make it practically impossible to administer the plasma through the standard types of filters used in administration sets. This will also be true when plasma without added dextrose is stored at room temperature.

Preparation and Use of Frozen Plasma

The technique for preparing frozen plasma differs slightly from that for liquid plasma. The steps outlined below have been found to be satisfactory.

1. Plasma is pooled as before.

2. Cultures should be taken immediately if it is desired to preserve the maximum content of prothrombin and complement during storage in the frozen state. If, on the other hand, it is desired to place maximum reliance on the test of sterility so that the plasma may later

be thawed and stored for longer than 24 hours in the liquid state, the pool should be allowed to stand 24 to 48 hours at room temperature (60° to 80° F.) prior to taking the cultures.

3. The addition of the bacteriostatic agent is optional. (See p. 52.)

4. Aspirate plasma into the final containers. (See "Filling of Final Containers.") If plasma is to be frozen, the final container should probably not be filled more than three-fourths to five-sixths full, in order to reduce the danger of breakage.

5. The plasma should be frozen promptly after the final containers are filled. The pilot bottle of the lot should be cultured and stored as indicated in items 10 to 12 on page 53. The plasma should not be used until all cultures have been reported negative at the end of 10 days.

6. It is preferable to place bottles in a frame which tilts them slightly so that more surface area is available to accommodate expansion of the plasma during freezing.

7. A special low temperature cabinet should be used. These units are available commercially and are ordinarily used for quick-freezing and storage of frozen foods, etc. The plasma is stored in this freezing chamber until ready for use. A temperature range of minus 15° to minus 20° C. is required. Within this temperature range, it is possible that plasma may be stored indefinitely. *Above minus 15° C., labile constituents of plasma will slowly disappear.* Plasma should be completely frozen within 4 to 6 hours after it is placed in the cabinet. Certain cabinets now available incorporate a quick-freezing device, such as a fan to circulate the air, or a means of placing the bottles containing plasma in direct contact with the refrigerating walls. If such a device is not built into the cabinet, quick freezing may be accomplished by immersing the bottles in a container filled with a substance (e. g., alcohol) remaining liquid at 20° C.

Restoring Frozen Plasma to the Liquid State

Place the container in a regulation water bath adjusted to 37° C. It requires 20 to 30 minutes for the frozen plasma to thaw. If plasma has been rapidly frozen and is thawed at 37° C., there is no fibrinogen precipitation. It is desirable to have plasma available for immediate use. This may be accomplished by thawing one or several bottles of frozen plasma and retaining them as liquid plasma. All plasma should be filtered while being administered. This reliquefied plasma should be stored at room temperature until used. It now has the same storage characteristics as described previously for liquid plasma.

Preparation of Dilute Liquid Plasma

Considerable evidence exists that pooling is unnecessary in the preparation of dilute plasma (50 percent plasma and 50 percent

diluent). Plasma prepared by this method is low in protein content and is not as satisfactory for the treatment of some conditions as is undiluted plasma. (See p. 64, "Advantages for Blood Banks.") The diluent commonly employed is 5 or 10 percent dextrose in normal saline. While this method is probably less desirable than pooling after centrifugation, its simplicity and the minimum amount of equipment needed may prove advantageous to the small plasma unit doing only a few bleedings per week. The technique for this method is essentially the same as that described under "Pooling of Citrated Liquid Plasma," except that individual final containers are used instead of pooling bottles. The bacteriological cultures may be made by withdrawing plasma from the final container or by taking a sample during aspiration. Use 10 cc. from each container and place into two culture tubes. Incubate one at 37° C. and the other at room temperature. (See instructions for "Addition of a Bacteriostatic Agent.")

Before aspirating the plasma, check the serologic tests to make sure that they are negative.

1. Follow the instructions for opening the bottle containing the blood and preparing the final container (substituting in this case a 600 cc. bottle, containing 250 cc. of diluent, for the pooling bottle). There are given in items 1 thorough 5 on page 50.

2. Insert the needle of the airway filter through the rubber stopper of the blood bottle and release the vacuum.

3. Close the donor valve and plunge the donor valve needle through the stopper of the final storage container.

4. Insert the aspirating needle through the stopper and into the supernatant plasma.

5. Open the valve and start aspiration. The aspirating needle must be kept beneath the surface of the plasma to avoid loss of the vacuum in the final container.

6. A strong beam of artificial light should be focused at the junction of the buffy coat and plasma during the aspiration. This serves to aid in the detection of cellular elements that might be drawn into the aspirating needle. Care must be taken not to aspirate any of the red cells. Do not attempt to aspirate all of the plasma, or a number of red cells will be pulled over into the final container. Aspirate the last 30 to 40 cc. of plasma very slowly to prevent lifting and drawing over the cells. Do not attempt to aspirate the last 10 cc. of plasma. It is possible to use the aspirating set more than once, and specimens for culture may be taken from the aspirating set during transfer to the next container. (See p. 66, "Culturing the Plasma" and "Multiple Aspiration.") *Handle bottle with care to avoid agitation of the red cells.*

2. EMPLOYMENT OF REUSABLE EQUIPMENT

While some of the equipment now commercially available is entirely satisfactory for the production of plasma, many hospitals prefer to prepare their own equipment. This is particularly desirable in institutions having well equipped laboratories, in view of the fact that it makes possible a considerable saving.

The technique described here employs gravity for collection of the blood; it is simple and economical; and it requires minimal replacement of critical rubber parts. It can be used for separation of plasma by centrifugation or sedimentation, and maintains a closed system throughout the process (38, 39, and 40).

For selection, preparation, and care of the donor during bleeding, and the storage of blood and of plasma, follow the instructions given elsewhere in this manual. (See pp. 4–5, 28–34, 52–55.)

Apparatus for Collection of Blood

The following parts are required for the assembly of the apparatus (see fig. 2) for collection of blood:

1. A glass bottle made of high grade hard glass, 9.2 cm. in diameter (3⅝ in.), 16.6 cm. in height (6⅝ in.), and with a capacity of approximately 650 cc. This bottle will fit standard centrifuge cups, and is so built as to withstand high speed centrifugation (2,000 to 2,500 r. p. m.). The neck is short and has an inside diameter of 26 mm.

2. A hooded, two-hole rubber stopper, fitting the bottle described above.

3. Two pieces of glass or stainless steel tubing (*B* and *C*) 7 mm. outside diameter, 3 and 4 cm. long respectively.

4. Two pieces of transparent amber rubber tubing, 8 cm. long with an outside diameter of 0.48 cm. and a wall thickness of 0.16 cm. (*G* and *E*) connected to tubes *B* and *C*.

5. One air filter (*F*) consisting of a glass tube 6 mm. outside diameter and 5 cm. long, with both ends slightly closed by flaming. This tube is filled with cotton, and connected with rubber tube (*G*).

6. One glass window (*H*), consisting of a glass tube 5 cm. long and 6 mm. outside diameter, connected with rubber tube *E*.

7. One piece of rubber tubing (*I*) of the same size mentioned under 4 above, 60 cm. long, connected to glass window (*H*).

8. One bleeding needle (*J*), 5.62 cm. long (2¼ in.), gage 15, with a round hub to fit rubber tube (*I*). The needle is protected by a 15 by 100 mm. glass tube firmly fitted with cotton around the hub of the needle (*J*).

9. A plain rubber stopper to fit the 15 by 100 test tube.

10. A cloth pocket, with tapes for tying, to hold the 15 by 100 test tube.

After proper cleaning of the glass and rubber parts, 50 cc. of sodium citrate solution is introduced in the bottle. The formula is as follows:

Sodium citrate (dihydric)—38 grams Sodium chloride—8.5 grams Citric acid—47.5 milligrams	water to 1,000 cc.

The set is then assembled as shown in figure 2. Care is taken so that tubes (*B*) and (*C*) project exactly 12 mm. above the hooded rubber stopper. Rubber tubes (*G*) and (*E*), when fitted to tubes (*B*) and (*C*), must cover their entire exposed portion and be in contact with the hooded rubber stopper. This permits some tendency to adherence to the stopper after sterilization and prevents slipping off later on.

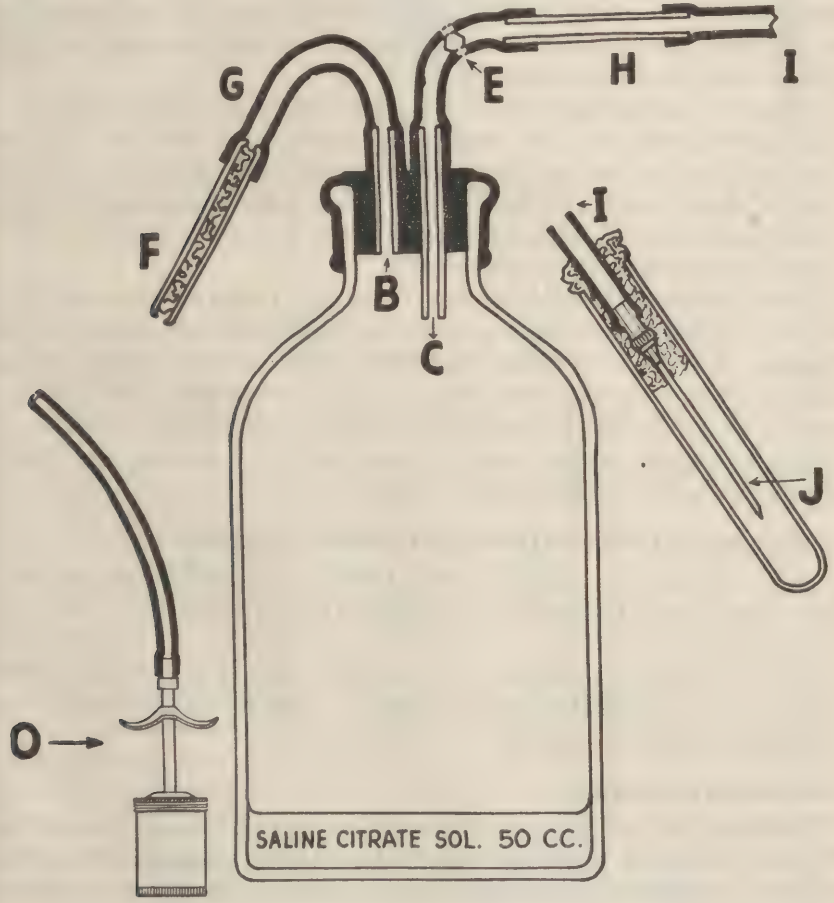

FIGURE 2.—Apparatus for the collection of blood.

The assembled set is placed in a bag of unbleached muslin with the small rubber stopper. The bag is closed, and the apparatus steam-sterilized at 121.5° C. for 20 minutes.

If the set is to be transported outside of the institution, it is desirable to close the rubber tubes (*G*) and (*E*) after sterilization. This is accomplished by clamping them through the bag with a stout pinch clamp.

Collection of Blood

During the collection of blood, the bottle is maintained well below the height of the patient's arm, as far below as permitted by the length of rubber tube (*I*). Generally, blood flows well by gravity, but initially the flow may be started by suction with a small hand pump (*O*) applied to air filter (*F*). As soon as the blood flows freely, remove the hand pump. During collection of blood the bottle should be agitated by a gentle rotating motion, to insure thorough mixing with the sodium citrate solution.

When the full amount of blood has been collected, release the tourniquet, pinch tube (*E*), and remove the needle from the vein. Place the needle inside the test tube, and by gentle milking, discharge 5 to 6 cc. of blood from the rubber tube. The test tube is immediately stoppered, and stored in the cloth pocket. This is tied to the neck of the bottle along with an identification tag.

Next fold down the rubber tubes (*G*) and (*E*) with a slight pull, and fix them tightly against the neck of the bottle with two stout rubber bands. This effectively seals the bottle. (Excessive stretching of the rubber tubes will cause poor closing of the rubber about the cannula used for drawing off plasma, thus allowing entrance of unfiltered air.) After sealing the bottle, mix the blood well for 1 minute, and place the bottle in the refrigerator at plus 2° to 5° C.

Separation of Plasma from the Cellular Elements

If plasma is to be separated from the blood by centrifugation, this procedure should be carried out within 72 hours, preferably within 24 to 36 hours.

If sedimentation is resorted to, the blood should be allowed to remain in the refrigerator for 6 to 7 days. (See following sections on "Sedimentation Methods".)

Pooling Apparatus

Drawing off, pooling, and distribution into individual containers by a closed system may be accomplished with the apparatus shown in figure 3. Essentially, this consists of a pooling bottle (1), an aspirat-

ing cannula (2), and a distributing cannula (3) assembled as a single unit. The apparatus is steam-sterilized at 121.5° C. for 20 minutes and effectively safeguards sterility of the plasma. Before sterilization, place about 5 cc. of freshly prepared distilled water in the bottle (1). The pooling bottle (1) should be of a capacity proportionate to the number of bleedings. For a pool of 10, a 4-liter bottle is satisfactory.

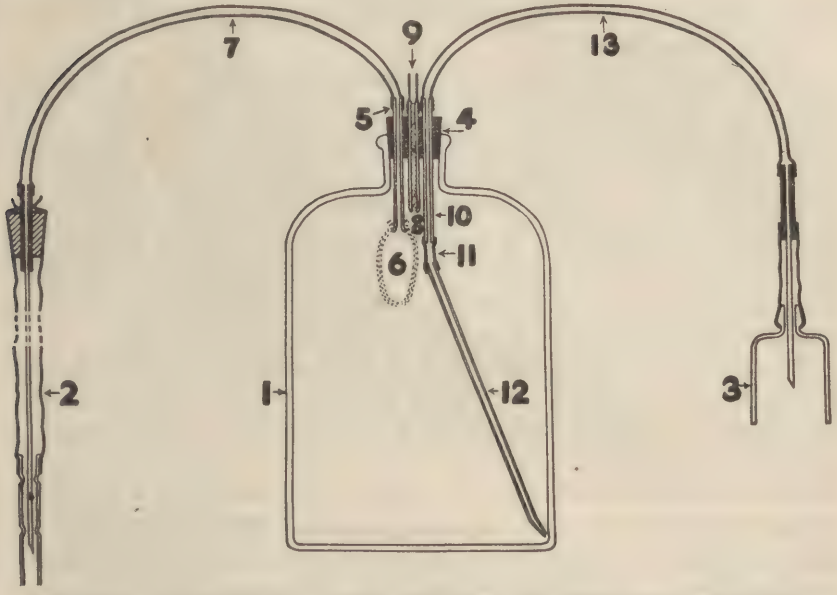

FIGURE 3.—Apparatus for pooling, filtration, and distribution of plasma.

The bottle is closed with a three-hole rubber stopper (4). Through one hole passes a piece of glass tubing (5) 10 cm. long and 7 mm. outside diameter. The lower extremity of this tube is flanged, and to it is solidly tied a bag (6) which acts as a filter. This bag is made of four layers of 40-mesh gauze and should be at least 5 cm. long. After preparation of the filter, it is boiled for 5 minutes in freshly distilled water, rinsed once or twice, and rapidly air-dried. This tube (5) is connected by means of a transparent amber rubber tube (7) about 60 cm. long, to the aspirating cannula (2). Through the second hole passes a glass tube (8) 7 cm. long and 7 mm. outside diameter. The ends are slightly closed by flaming, and the lumen is filled with cotton. This tube acts as an air filter; to it is attached the rubber hose (9) connecting the bottle with the vacuum pump. Through the third hole passes a glass tube (10) 10 cm. long and 7 mm. outside diameter. The lower extremity is connected by means of a short piece of rubber

tubing (11) to a long glass tubing (12) which reaches to the bottom of the pooling bottle. The lower extremity of this tube should be slightly bent and drawn so as to reach the angle that the bottom of the bottle forms with the sides. The upper extremity of the glass tube (10) is connected by a transparent amber rubber tube (13), approximately 60 cm. long, with the distributing cannula (3).

The plasma is drawn off the blood through the large bore cannula (2) which perforates one of the rubber tubes of the donor bottle. The rubber tubes (*E*) and (*G*) stretched across tubes (*B*) and (*C*) form perforable membranes.

The cannula is of 18–8 stainless steel seamless tubing. It is 15 inches long, gage 13, outside diameter 0.095 inch. The inside diameter is 0.073 inch; the wall is 0.011 inch in thickness. The hub which makes connection with the rubber tubing is of brass, nickel-plated, and 2 inches long. The cannula terminates with a sharp bevel, but the bevel is closed and the opening is 4 mm. from the end. This permits the cannula to perforate the rubber tube of the blood bottle and the tip to touch the buffy coat of leukocytes without danger that the cellular elements will be aspirated from the plasma as it is drawn off.

Because the aspirating cannula will go in and out of several bottles of blood, it is necessary to protect it from possible contamination. This protection also allows indirect handling of the cannula, which is quite important technically from the standpoint of ease of operation. Protection is accomplished by means of a rubber sleeve and a glass bell. The protecting sleeve of the cannula is made of thin black rubber tubing, outside diameter 1 inch flat, commercially known as Gooch crucible tubing. It can also be made of a cellophane tube. The glass bell is made of a piece of Pyrex glass tubing 9 cm. long with an outside diameter of 1.5 cm. The upper end is flanged to allow firm attachment to the rubber sleeve. There are two constrictions dividing the bell into approximate thirds where the lumen is reduced so as barely to permit passage of the steel cannula. This arrangement centers the cannula and keeps it from touching the sides.

To assemble the component parts of the aspirating cannula, the hub of the cannula is first pushed through a No. 2 rubber stopper and then attached to the rubber tube leading to the pooling bottle. Next the protecting rubber sleeve is slipped over the cannula and rubber stopper and the proximal end tied firmly around the hub. The distal end of the sleeve is tied to the glass bell. The length of the sleeve is such that the opening of the bell extends beyond the tip of the cannula by about 2 cm. The open end of the bell is loosely stoppered with cotton and covered with a paper cap before sterilization and remains so until ready for use.

When this apparatus is used for drawing off the plasma, it is important to use an air filter. This is made of an ordinary intravenous

needle, gage 19 or 20, about 1 inch long, the shank of which is connected by a short piece of rubber tubing (2–3 cm. long) to a 4 or 5 cm. length of glass tubing, about 7 mm. outside diameter. The ends of this tube are turned in slightly, and the lumen is filled with cotton. The whole filter is steam-sterilized. The filter is used to allow air to enter the blood bottle as the plasma is drawn off. Its needle perforates the rubber tube not punctured by the aspirating cannula.

The distributing cannula (3) is similar to the one used for aspirating, but it is shorter and the opening is at the tip. The length of the stainless steel cannula and the size of the glass bell vary according to the size and shape of the final container. For the standard 400-cc. bottle, the cannula is 5 inches (12.5 cm.) over-all in length. The glass bell has a diameter of 3.5 cm. at its base and a depth of 4.5 cm. The protective sleeve covers the upper ⅔ of the cannula and is of soft black gum rubber tubing ¼ inch inside diameter with a $\frac{3}{64}$ inch wall. Its lower end is tied to the bell, and the upper end slips over the lower part of the hub of the cannula. The upper part of the hub is connected to the rubber tube (13). The protective sleeve is of appropriate length to keep the tip of the cannula high within the bell. The thin wall of the sleeve folds up and easily allows shortening as the cannula is pushed through the stopper of the final container. The entire bell and distributing cannula are wrapped before sterilization.

Pooling of Plasma

To draw off the plasma, proceed as follows: The work should be done in a closed room, preferably in a cubicle, by operators wearing cap and face mask. The installation of properly placed sources of ultraviolet radiation, such as a germicidal lamp, may aid in reducing contamination, but is not essential. The installation of such sources of ultraviolet radiation must not encourage false confidence. The tubes marked (7), (9), and (13) in figure 3 are provided with screw clamps. A suction bulb, or better, a vacuum pump, is attached to the air filter (8) through tube (9). The heads of the blood bottles are carefully prepared by covering for 10 minutes with gauze saturated with 10 percent cresol compound solution. When the gauze is removed, it is replaced with a sterile alcohol sponge (or iodine and alcohol may be used, as described on p. 50). Diaphragms formed by rubber tubes (*G*) and (*E*) stretched over the tubes (*B*) and (*C*) are similarly prepared. The bell protecting the aspirating cannula (2) is unwrapped, unstoppered, and placed over the rubber diaphragm (*E*) or (*G*). The cannula, protected by the rubber sleeve, is then grasped near the hub and pushed down into the blood bottle until it reaches the plasma. The rubber sleeve folds up after the manner of an accordion as its length is shortened. An air vent and filter are

pushed through the diaphragm of the other tube, to allow entry of filtered air to replace the plasma being drawn off.

The next step is to release the screw clamp on the tube marked (7) on figure 3 and allow the plasma to be sucked into the pooling bottle. Aspiration is carried out until all but a thin layer of plasma is removed. Advantage is taken of the viscid buffy coat of leukocytes and fibrin to prevent stirring of the red blood cells. Since the air is filtered, some may safely be allowed to enter the cannula when the last few drops of plasma are aspirated. This enables the operator to obtain a greater yield. The amount of plasma which cannot be conveniently removed averages 20 cc., of which 5 cc. are saline-citrate solution. This loss will be kept quite constant by skilled manipulation, which comes with experience. (See p. 51 for use of artificial illumination.)

When the operation is finished, the cannula is raised back into the sleeve so that the tip is well within the bell, the mouth of the bell is flamed, and the entire procedure is repeated on the next bottle, until all plasma has been drawn off. With some experience, technicians may handle 24 bottles in 1 hour. However, to reduce loss from accidental contamination, it is advisable to limit the pools to 10 bleedings. In cases in which more than 10 are available, additional pools of convenient size are made using separate pooling bottles. One hundred cubic centimeters of 50 percent dextrose should be added for each 900 cc. of pooled plasma. (See pp. 46 and 50.)

Culturing

In the previous technical description of "Culturing the Pool," it was indicated that specimens of plasma were to be inoculated into *culture tubes*. This technique requires careful stoppering of the tube, dust-free storage, and extreme care in opening for inoculation, if accidental contamination is to be avoided. The following paragraph describes the use of bottles, closed with a perforable rubber stopper, for the culture medium. The use of this technique is most satisfactory, as it almost entirely eliminates the danger of contaminating the medium at the time of inoculation.

When the desired number of specimens are pooled, close the screw clamps of tubes (7) and (9), leave the cannula in the last blood bottle to preserve its sterility, and mix the pool by rotating the large bottle gently. (See page 53, "The Preparation of Frozen Plasma," for the proper time interval to allow before taking the specimens for culture.)

The next step is to obtain a specimen of the pooled plasma for cultures as well as total protein determination, and any other examination desired. The introduction of plasma into the bottles containing culture medium and the sample bottle is made possible by a preformed vacuum in these bottles, effected before sterilization. Tube (9) is detached from (8), which acts as a filter. Carefully unwrap the

mouth of the distributing glass bell; apply it over the sterile perforable rubber stopper of a bottle of culture medium; lower the distributing cannula and perforate the rubber diaphragm; release the screw clamp of tube (13) and introduce approximately 10 cc. of the citrated plasma into the medium; withdraw the cannula and repeat with three other bottles containing 20 cc. of thioglycollate medium. Specimens for other desired tests are obtained in a similar manner, but the collecting bottle contains no medium. For further details see "Culturing the Pool," page 51, and appendix B, section IV, page 82.

Addition of a Bacteriostatic Agent

After the necessary samples are obtained, the preservative may be added, if desired. (See p. 52.) The following technique is recommended for reusable equipment:

The measured amount of preservative is contained in a bottle or test tube closed with a sterile perforable rubber stopper. The distributing steel cannula (3) is introduced through the perforable rubber stopper into the preservative solution. Tube (7) is clamped shut, and a vacuum is created through tube (9). A sterile needle with a cotton filter allows entrance of filtered air into the bottle containing the preservative. Thus the preservative is added to the pooled plasma with maintenance of the closed system.

Filling of Final Containers

1. *Preparation of the storage bottle for use.*—The recommended standard bottle is cylindrical, of low solubility glass blown in a mold, and of a capacity of a little over 400 cc. The body is 72 mm. outside diameter, the length over-all 157 mm., the neck 22 mm. long, with a tapered bore 12 mm in diameter. The thickness of the walls is 2.5 mm. The bottle should weigh not less than 210 grams. A heavy lip allows tight fitting of a hooded amber rubber stopper.

After suitable cleaning, about 1 cc. of freshly distilled water is introduced in the bottle, and the mouth closed with a hooded rubber stopper. A vacuum is then formed by means of a thin needle, introduced through the solid portion of the stopper, connected to a good water pump. The stopper is then covered with a cap of thick paper securely fastened to the neck of the bottle, and the whole is steam-sterilized at 120° C. for 20 minutes. The small amount of water in the bottles permits formation of steam, necessary to proper sterilization. The vacuum lessens the chances that the stoppers will be blown off during sterilization, and makes possible the introduction of plasma into the bottle by a closed method.

2. *Procedure.*—For distribution of the pooled plasma, the screw clamp of tube (7) is left closed. (Tube (9) is still detached from tube (8), which acts as a filter.) The final container is made ready

by removing the paper cap protecting the sterile perforable rubber stopper. The distributing cannula is forced through the sterile stopper by grasping the hub and directing the steel cannula through the rubber stopper. Release the clamp of tube (13) and allow the container to fill.

Experience has shown that if the pools are sufficiently large (2,000 to 3,000 cc.) and the individual blood specimens not too small (average 460 cc. excluding the sodium-citrate solution), the average total protein content of the citrated plasma is 6 grams percent. Approximately 300 cc., therefore, contain 17.5 grams of plasma proteins. The bottles into which the plasma is distributed are graduated or marked at 300 cc., and plasma is allowed to flow to the mark.

In filling the final containers, a pilot bottle should be prepared and cultured as directed in paragraphs 10, 11, and 12, page 53.

B. Sedimentation Methods

In the technical descriptions of the centrifuge method details were presented for both commercial vacuum containers and reusable hospital equipment. In the following presentation of techniques, details are given only for the commercial vacuum bottles. The reader can easily adapt reusable equipment to these methods by following the principles and techniques described for the centrifuge method.

1. BLOOD PRESERVATION AND SEDIMENTATION METHOD FOR THE PREPARATION OF DILUTE BLOOD PLASMA

This technique involves the use of a dextrose preserving solution and offers advantages if a blood bank is to be maintained (14). A satisfactory dilute plasma can be prepared with a minimum of expense, equipment, and technical effort. Three such solutions have been described in Part I of this manual.

Advantages for Blood Banks

During the period of storage the blood is always available for use as whole blood, and the preservative enables the freshest blood to be used rather than the oldest—for blood does not become outdated, in the sense that it must be discarded; it merely becomes ready for the aspiration of plasma. This method is advantageous in the operation of a whole blood bank and results in the preparation of a dilute blood plasma with a yield which is only slightly less than that obtained by centrifugation. The effectiveness of dilute plasma in the treatment of shock has been well demonstrated clinically and experimentally. Its chief disadvantage is that more fluid must be stored for each unit of plasma. Dilute plasma is not as desirable as isotonic

plasma in some cases; for example, in cases of injury producing cerebral edema or those in which there has been a large loss of blood protein, as in untreated (severe) shock and in severe burns.

Blood Preservation Technique

The donor is selected and prepared for venesection, and the blood is drawn into the flask containing the preserving solution. (See "The Technique of Collection," p. 30.) At the close of the bleeding, the blood remaining in the donor set is utilized as follows, because the collection is to be stored for potential use as whole blood:

Two or three drops of blood are allowed to flow into a small test tube containing 3 to 5 cc. of the preservative solution. The remainder of the blood is put into the serology tube. Before this clotted blood is used for the serologic tests, two or three capillary pipettes (drawn from glass tubing) are filled with serum, sealed, and placed in the tube containing the cell suspension. This tube is clearly marked and attached to the bottle containing the blood for use in grouping and cross-matching.

The flask of diluted blood is now gently but thoroughly mixed again, labeled, and placed *at once* in the refrigerator at 2° to 5° C. It remains there in storage until used as whole blood or until the plasma is aspirated at the end of the storage period. Although the time required for sedimentation is about 16 days, if a maximum yield of plasma is desired, and should be at least 10 days to be sure that all red cells have settled out, it is possible, in an emergency, to obtain a fair amount of plasma after as little as 48 to 72 hours of sedimentation. Such plasma will still contain some red cells, but not a sufficient quantity to be dangerous, even if transfused into an incompatible recipient. Experience indicates that the transfusion of from 75 to 100 cc. of incompatible whole blood seems to be required to produce a severe type of reaction.

Aspiration of Plasma

During this time the serologic tests will have been done. The flask is removed from storage for aspiration of the supernatant plasma layer. The cover is removed aseptically, and the stopper cleaned with iodine and alcohol. The vacuum is released by placing an air filter through the stopper. The "air tube" outlet is not disturbed, for the passage of any air through it would at once stir up the settled red cells. An 18-gage instead of the customary 20-gage needle is used on the air filter, because it is strong enough to puncture the rubber stopper easily. An aspirating set with a 6-inch needle is opened (the needle should be covered by a penrose tube as described before). The closed valve is inserted in an empty 600 cc. container. The long needle is placed

through the stopper so that the point is about 1 inch below the surface of the plasma, and the aspiration is begun. At first it can be carried out quite rapidly, but when the fluid level gets to within an inch of the red cell layer, the rate of flow should be slowed to avoid stirring up the settled fibrin and red cells. The end of the needle must always be kept below the surface of the plasma, for, if much air is allowed to replace the vacuum in the 600 cc. flask, the aspiration cannot be completed. The valve is closed as soon as the desired amount of plasma has been obtained, and the aspirating needle is pulled above the fluid level. About 20 cc. is left to avoid the danger of aspirating red cells into the plasma. (See also pp. 54–55.)

Culturing the Plasma

A culture is now taken from the plasma remaining in the aspirating set by withdrawing the donor valve and allowing 4 to 6 cc. of plasma to run into each of two culture tubes containing thioglycollate medium. These are prepared and cultured as described previously for dilute plasma (p. 55).

Multiple Aspiration

It is possible to draw plasma from more than one flask with the same aspirating set without danger of contamination. At the end of the first collection, the valve is closed and the end of the aspirating needle pulled up slightly to avoid contact with the red cells. The second flask of blood is prepared as described, using the air filter taken from the first one. A second empty 600 cc. flask is opened aseptically and the top prepared as described, with iodine and alcohol. Now, the closed donor valve is removed, cultures taken as just described, and the valve at once placed through the stopper of the empty 600 cc. flask. Now the aspirating needle is transferred, after the excess iodine and alcohol have been wiped from the top of the new flask of blood. The collection of plasma proceeds as before. With this method there is minimum handling of the aspirating set; the needles touch only the sterile stoppers of the flasks, and their exposure to room air is only momentary. This procedure has been found satisfactory. It saves time in making multiple collections and in maintaining the equipment. An aspirating set should probably not be used more than 10 times without resterilization.

Addition of a Bacteriostatic Agent

This procedure is optional. Refer to page 52 for discussion and method.

Use as Liquid Plasma

After the cultures have proved sterile (10 days as described previously), the plasma is ready for use. It is preferable to store liquid

plasma at room temperature rather than at 2° to 5° C., because less precipitate will form. However, in plasma made by this method the amount of precipitate is considerably less than in that made by the centrifuge method. It must be remembered that this plasma is dilute.

2. CITRATE SEDIMENTATION TECHNIQUE

This method consists of collecting blood in citrate solution in the same manner as is done for an immediate transfusion of whole blood or for blood that is to be centrifuged to prepare plasma. There are several variations in the shapes of collection bottles now available commercially; some of them provide a small diameter interface between settled red cells and the supernatant plasma, thus permitting a slightly greater yield. The use of this method, however, results in a yield of plasma which averages 30 percent less than that obtained by the centrifuge method. Unless the following technique is meticulously followed, the plasma obtained by this method will be unsatisfactory because of excessive clot formation during storage of the plasma.

1. During the collection of blood from the donor, the operator must be particularly careful to insure complete and thorough mixing of the blood with the citrate solution. The presence of clots in the collected blood greatly enhances subsequent clotting of the plasma. The technique is described on pages 47–49.

2. When the collection of blood is completed, the flask is stored in a refrigerator maintaining a temperature between 2° and 5° C.

3. The blood is allowed to sediment, preferably for 7 days. This settling period should never be less than 4 days and should not exceed 7. Clearer plasma as well as a larger yield will be obtained by using the 7-day period.

4. At the end of the storage period the flask of blood is carefully removed from the refrigerator, and the plasma is aspirated into a 2,000 cc. pooling bottle containing 200 cc. of 50 percent dextrose (see p. 50). After the pooling has been completed, cultures should be taken as described on page 51. The addition of a bacteriostatic agent is optional (see p. 52).

5. The pools of plasma are then allowed to remain at room temperature for the 10-day period during which the cultures are incubated. This is important because it permits further sedimentation, which results in a more satisfactory product.

6. After the cultures have been checked and found negative, the pool is ready for distribution into final containers. The pooling bottles should be handled carefully to avoid stirring up the sediment.

Final containers are filled according to the instructions given on pages 52 and 53. The pilot bottle must be prepared and cultured as described on page 53.

7. This plasma should be stored either at room temperature or in the frozen state. Directions for freezing and thawing are given on pages 53 and 54.

8. This entire procedure must be carried out with the use of a closed system.

Chapter IV. *Labeling of Plasma*

Proper labeling of the final plasma container is essential in order that the clinician may know exactly how much original plasma, diluent, and preservative he is administering. The plasma label should contain the following information:

1. Name and address of the laboratory preparing the plasma.
2. Lot number or pool number.
3. Date prepared.
4. Amount and kind of diluent added.
5. Amount of original plasma.[7]
6. Total protein content of the final container (optional; however, each laboratory should determine its average standard for the method used).
7. Amount and kind of preservative, if added.
8. Date and results of aerobic and anaerobic cultures (thioglycollate medium).

Chapter V. *Plasma Records*

The records of the plasma unit of any institution must be accurate, complete, and duly signed in order that they may constitute a legal record should it ever be necessary to use them as such.

These records should include the following data:

1. The number, name, address, history, and physical examination of the donor.

2. A statement signed by a physician that the donor is free from diseases transmissible by blood or plasma transfusion.

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[7] Original plasma is defined as the liquid portion of the blood as it comes from the veins of the donor, before dilution with the anticoagulant; e. g., 350 cc. of citrated plasma is obtained from 550 cc. of blood and if 50 cc. of anticoagulant has been added in collecting the blood, the amount of original plasma is considered 300 cc. In other words, original plasma equals total amount of citrated plasma minus the amount of sodium citrate solution used in collecting blood and dextrose solution added in pooling.

3. A release, signed by the donor, authorizing the bleeding.
4. The date and amount of blood collected from the donor.
5. Voluntary or paid donor.
6. The results of recognized serologic tests for syphilis.
7. Blood grouping is recommended but not required.
8. Names or the serial numbers of the donor's plasma, constituting a lot or pool number, and indicating the technique of preparation and storage.
9. Dates and number of bacteriological cultures.
10. Recipient's name and date of administering plasma.
11. Record of any unfavorable reactions.

Chapter VI. *Care of Equipment*

Preparation of Equipment for Intravenous Use

Up to the present time, all intravenous equipment has depended on the availability of high-grade gum rubber tubing manufactured especially for this purpose. This implies that the maker has taken precautions to eliminate soluble and insoluble substances which would be unsuitable for intravenous injection. This type of rubber must be regarded as perishable, and certain facts should be recognized. The rubber deteriorates particularly through oxidation. This process is accelerated by high temperatures and the presence of moisture. The reserve stock should be kept in a cool, dark, dry place. It is advisable about once a month to stretch each piece several times by hand.

Gum rubber deteriorates very rapidly with each autoclaving. Extensive experience with one make of latex rubber tubing has shown that it will stand about 15 sterilizations with steam heat before it is rendered unsuitable for intravenous use by loss of elasticity and increased cohesiveness. Another fact is noteworthy. When gum rubber is heated in the presence of moisture, it takes on a cloudy appearance which is due to combination with the water. This disappears when the rubber is thoroughly dried either in the autoclave or in the air. While this state exists, the rubber is extremely delicate and is much more susceptible to injury. The life of the tubing may be prolonged if care is taken to have it thoroughly dried before using. This may be accomplished by drying in the autoclave after sterilization or by allowing the sterilized packages to stand for several days before using. New tubing, unless expressly prepared at the factory, must be specially treated before use. (See appendix A, sec. III.)

The elimination of pyrogens is perhaps the most difficult and the least understood procedure in the preparation of equipment for in-

travenous use. Many species of water bacteria may grow for some hours in distilled water and produce soluble, ultrafilterable substances which cause chills and fever when injected intravenously into rabbits or into man. These substances are not inactivated by the heat used in the sterilization of equipment. Pyrogens can be washed out of equipment by rinsing with pyrogen-free fluids. It is necessary, then, to have a source of pyrogen-free water with which to prepare equipment. This may be obtained commercially from the firms manufacturing fluids for intravenous use, or it may be manufactured in the laboratory by the efficient distillation of water from the sources utilized for drinking water. A good single still (a triple still is not necessary), if run at less than full capacity, will furnish pyrogen-free water. Water may be tested for the presence of pyrogens by the intravenous injection of samples into rabbits and observing their body temperature over a few hours. For details, see appendix B, section VII. After the equipment is mechanically and chemically clean, it is rinsed with pyrogen-free water. *It should then be dried rapidly or wrapped and autoclaved before time has elapsed for bacteria to grow in the resulting moisture.* (Glassware may be dried in a laboratory or domestic oven. The rubber tubing may be dried by forcing dry, clean air through it.)

The mechanical removal of blood, serum, and dirt may be accomplished by forcing tap water (if low in pyrogens; otherwise, use freshly distilled water) through the tubing and then washing with hot water containing a detergent such as trisodium phosphate. Coagulated protein may be forcibly removed from rubber tubing by inserting pipe cleaners, which may be obtained from laboratory supply houses in 3-foot lengths or use a length of wire and gauze, as described in the following section.

An outline of the procedures for the preparation of equipment for intravenous use is as follows:

1. Soak soiled equipment in cold water for several hours to remove some of the blood and plasma proteins.
2. Clean glassware and tubing mechanically with brushes and pipe cleaners, again using cold water.
3. Wash in warm tap water to which has been added a detergent such as trisodium phosphate.
4. Rinse thoroughly with warm tap water. The alkaline trisodium phosphate clings to surfaces tenaciously and requires much washing to remove.
5. Rinse at least twice with pyrogen-free water.
6. Assemble intravenous equipment, package, and autoclave, preferably at once if wet, or within a few hours if equipment has been dried before assembling.

Cleaning and Preparation of the Donor Sets

All donor sets must be thoroughly cleaned and autoclaved *within 4 hours* after use to prevent bacterial growth from forming in the tubing.

1. Immediately after use, disassemble the set and thoroughly flush all parts with cool, fresh distilled water (cold tap water may be used if it is known to be essentially free from pyrogens). It is important that this step be carried out within a few minutes after use.

2. The donor valve needles must be thoroughly cleaned. This may be accomplished by attaching a heavy string to a thin piece of wire and pulling it through the needle several times. If blood has been allowed to clot in the needles, they should be allowed to soak in hydrogen peroxide for a short time before they are cleaned with the wire and string.

3. The translucent rubber tubing should be thoroughly washed with physiological saline and inspected against a bright light for evidence of clotted blood. If clots are noted, they should be removed by rolling the tube between the fingers. Flush the tubing with normal saline, and then a wire with hydrogen-peroxide-saturated gauze attached should be pulled through the tube several times. Following this procedure, flush the tubing again with normal saline. Treatment with a detergent may be used in place of peroxide.

4. Cut off approximately one-half inch of each end of the rubber tubing. The ends of the tubing in contact with metal lose their elasticity as a result of the excessive heat during autoclaving. Cutting off the ends of the tubing following use assures a tight, leak-proof fitting.

5. Commercial donor valves should be cleaned and reassembled in accordance with the specific instructions furnished by the manufacturer.

6. Before placing the set in the tray for autoclaving, flush tubing, needles, and valve with clean, pyrogen-free normal saline to prevent hemolysis from occurring when the set is next used.

7. Put small test tubes over the donor needle and valve needle.

8. Place the donor set in the tray for autoclaving. Care should be taken not to allow the rubber tubing to come in contact with the metal parts, as it will cause the tubing to adhere and blister, rendering it unfit for further use.

Note.—Preparation of the Donor Tray: A standard metal or wire tray covered with muslin may be used as a container for the equipment used in collecting blood. Each donor tray should contain:

1. One donor set (valve, tubing, 17- or 15-gage donor needle).
2. One 2-cc. syringe.

3. Two hypodermic needles, 26-gage.
4. Six 2- by 2-inch sponges.
5. One Diack control to assure sterilization (for perfect control).
6. Two serology tubes.

The tray should be adequately wrapped in muslin and tied. Do not use pins, as they may pierce the rubber tubing.

Autoclave the tray for 30 minutes at 15 pounds pressure (start timing after the pressure has reached 15 pounds).

Reautoclave the tray if not used within 10 days. A heavy cloth wrapping for these sets may be used without the tray if desired.

Cleaning and Preparation of the Aspirating Set

The aspirating set consists of the same equipment used for collecting blood from the donor, except that a 6- or 9-inch aspirating needle is used instead of the donor needle. In order to maintain sterility, it is essential that the aspirating needle be protected by covering it throughout its entire length with a penrose tube which is tied in place over the hub of the needle. The aspirating needle should be well glycerinated in order that the penrose tube may slide up and down easily as the needle is inserted into and withdrawn from the bottle. A short length of glass tubing should be attached to the end of the penrose tube so that it extends over the tip of the needle.

When plasma, pooled in a suitable 2,000-cc. bottle, is aspirated into the final dispensing bottles, it is usually necessary to use a 15-inch aspirating needle. This needle must also be protected with a penrose tube covering.

The aspirating set must be cleaned immediately after use by thoroughly flushing several times with freshly distilled water, cleaning carefully as described for the donor sets, and finally flushing with normal saline prepared from pyrogen-free, freshly distilled water.

After the aspirating set has been thoroughly cleaned, it is placed in a tray or wrapper similar to the tray used for the donor set. The aspirating set should be autoclaved (15 pounds pressure for 30 minutes) *within 4 hours* after use in order to prevent the growth of bacteria which may produce pyrogens.

Cleaning of Storage Bottles

The ordinary cleaning process routinely used in preparing for reuse bottles used for intravenous solutions, is not sufficient when the bottles have been used for blood or plasma. In this case, each bottle must be cleaned by nitric acid (concentrated), "cleaning solution," or one of the newer detergents especially prepared for this purpose, and then thoroughly rinsed in addition to the "regular" cleaning.

Chapter VII. *Adverse Reactions From the Administration of Human Plasma*

Adverse reactions from the administration of plasma may be due to any one or a combination of the following:

1. The use of impure sodium citrate, dextrose, or sodium chloride in the preparation of solutions used in bleeding, pooling, etc.
2. Pyrogenic substances in the distilled water used in making up the sodium citrate solution or in the cleaning of equipment.
3. Bacterial contamination of the blood used in preparing the plasma.
4. Failure to filter the plasma during administration.
5. Contamination of the pooled plasma.
6. The presence of red cell stroma in the plasma.
7. Improperly cleaned equipment used in bleeding, pooling, or filling the final container.
8. Improperly cleaned intravenous equipment.
9. The use of nonfasting donors is in part responsible for urticarial or anaphylactoid reactions (lipemic plasma). However, the use of fasting donors will not entirely eliminate these reactions.
10. Improper desiccation of plasma.
11. Heating plasma prior to or during administration.

The listing of these possible causes of reactions due to plasma administration may cause the uninitiated to feel that unfavorable reactions are rather common, while the contrary is true. *If plasma is properly prepared, reactions should be well under 1 percent and not severe.*

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APPENDIX A

Section I

The Preparation of 1 Percent Aqueous Merthiolate Solution

1. Carefully weigh out 1 gram of powdered merthiolate for every 100 cc. of final solution desired.

2. Add the accurately weighed merthiolate powder to the desired quantity of sterile, pyrogen-free, freshly distilled water. It is imperative that the water be distilled within 4 hours of the time of adding the merthiolate.

3. Weigh out and add 1.4 grams of reagent grade sodium borate for each gram of powdered merthiolate. Add the borate to the 1 percent merthiolate solution. (Not essential.)

4. Autoclave the entire solution at 20 pounds pressure for 30 minutes.

5. As soon as cool, the 1 percent merthiolate solution is ready for use. It should be stored in the dark at 4° to 8° C.

NOTE.—A 1 percent solution of phenyl mercuric borate or nitrate is made in the manner outlined above in items 1, 2, 4, and 5.

Section II

Refrigeration of Blood

The storage of whole blood, regardless of the method of preparation, requires adequate refrigeration. The essentials of this include:

1. Adequate space to avoid overcrowding.

2. An "air-conditioned" type of cooling coil (this provides constant circulation of air, does not require defrosting, and therefore maintains a constant even temperature).

3. Maintenance of storage temperature at 2° to 5° C. (to do this the cooling coils should be of a larger size than are commonly installed in domestic refrigerators).

4. Avoidance of excessive vibration (the current practice of suspending the compressor unit on rubber and spring mountings is usually sufficient for this purpose).

While the ordinary domestic type of refrigerator can be used in case of necessity, it does not meet the ideal requirements and therefore will not be entirely satisfactory.

Section III

Preparation of New Rubber Tubing[8]

1. To insure against temperature reactions caused by chemicals, thoroughly rinse all new tubing inside and out to remove excess sulfur and soluble compounds from the rubber.

2. Dissolve 50 grams of sodium carbonate in each liter of freshly distilled water (2 ounces of washing soda per quart), the water not to be over 4 hours from the still. Then place the rubber tubing in this solution, starting with one end, and lowering the tubing into the container in such a manner that it becomes full of solution.

3. Place the container in a sterilizer for 30 minutes at 15 pounds pressure (121.5° C.), since the undesirable compounds are not removed at temperatures under 115.5° to 121.5° C. (240° to 250° F.). The disagreeable odor and the residue in the water will prove the advisability of this technique.

4. Vigorously rinse each section of tubing repeatedly, using a minimum of 200 cc. of freshly distilled water each time (the water to be not over 4 hours from the still).

5. Tubing is now ready for set assembly and autoclaving.

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[8] If new rubber tubing has been specifically prepared by the manufacturer for use in intravenous therapy (and this is so stated on the package) this preparation is not necessary.

APPENDIX B

The following material has been taken from the *Minimum Requirements for Unfiltered Normal Human Plasma* prepared by the National Institute of Health for the control of the preparation of plasma by *commercial firms* and from U. S. P. XII requirements. These excerpts have been selected because they will be of use to hospitals as reference material. *Statements conflicting with the text of the manual in reference to the techniques described or tests required should be ignored, since they do not apply to hospital practice.*

Section I

Interpretation of the Serology Test for Syphilis

"1. An acceptable serological test shall be one which is acceptable to the responsible authority in the city or State in which the processing laboratory is located.

"2. Any blood showing a 3+ or 4+ reaction shall be considered as unsatisfactory, and the plasma from such a blood must not be processed.

"3. Any blood showing a plus-minus, 1+ or 2+ reaction shall be checked by another method, preferably a complement fixation test. If the same degree of reaction, or less, is obtained the blood shall be considered satisfactory and the plasma shall be processed. However, if the retest method is a complement fixation test, then a 2+ reaction shall indicate an unsatisfactory blood and the plasma from such blood must not be processed."

Section II

Methods for the Determination of Hemoglobin

"1. The determination shall be considered sufficiently accurate if a colorimetric comparison be made with a standard prepared from hemolyzed blood from a person whose blood hemoglobin content has been determined previously in terms of grams of hemoglobin. The standard is prepared as a dilution of hemolyzed blood in plasma which

is free of all visible trace of hemoglobin color. Hemolysis of the blood is effected by first diluting the blood 1 to 20 with distilled water and waiting until hemolysis is complete.

"2. The blood hemoglobin in the person serving as the source of hemoglobin supply shall be determined by a method at least as accurate as can be made by a Sahli apparatus equipped with permanent color standards.

"These color standards shall have been calibrated during the course of preparation by the manufacturer, using the Van Slyke oxygen capacity method, the Wong iron method, or a method recognized as the equivalent. (Ref.—Am. J. Clin. Path. v. 3, p. 85 (1932); v. 4, p. 354 (1934).)

"3. For an accurate quantitative determination of the hemoglobin content of the pooled plasma either of the following methods may be employed, or one equally accurate:

"Ham, T. H., Arch. Int. Med., v. 64, p. 1271 (Dec.) 1939.

"Bing, F. C., and Baker, R. W., J. Biol. Chem., v. 92, p. 589, 1931."

Section III

Pooling, Tests for Sterility, Storage of Plasma

"PREPARATION OF THE PLASMA POOL

"10. If plasma is to be processed only to the liquid state and dispensed in this form, or if it is intended to be processed to the frozen state without subsequent drying, there shall be added to the pool prior to taking the sterility sample a sufficient amount of sterile 50 percent dextrose solution so as to give a 5 percent concentration of dextrose in the finished plasma. Dextrose shall not be added to the plasma which is to be shell frozen and subsequently dried.

"11. Immediately after withdrawing the sample for the sterility test a sufficient amount of a suitable preservative shall be added, except that phenol or a similar compound shall not be considered a suitable preservative. (At the present time the following preservatives and concentrations are considered suitable: For liquid or frozen plasma, phenyl mercuric borate 1:15,000 or sodium ethyl mercuric thiosalicylate (Merthiolate) 1:10,000.)

"TESTS FOR STERILITY

"12. Tests for sterility are required on the plasma before the addition of the preservative. At this point the tests *may* be made on the plasma from the individual bleedings *or* on a sample taken from the

plasma pool prior to the addition of the preservative. Sterility tests are also required on the finished product as contained in the finished dispensing unit.

"13. *Test culture medium.*—The National Institute of Health has adopted as the standard culture medium for making the sterility test on all biologics under its control a medium designated as 'Fluid Thioglycollate Medium.' The Institute has available a memorandum covering the four recognized formulae. However, experience has shown that of these the Linden formula is the simpler and also more economical and is therefore recommended. (See sec. IV for the formula.)

"14. *Sterility tests on individual bleedings.*—When plasma is to be processed to the liquid or frozen state, the sterility test may be made on the individual bleedings. The following procedure shall be followed: The plasma shall be drawn off into separate individual containers through a closed system and retained in these containers until the sterility test is completed. The test sample shall be withdrawn from these individual plasma containers. The amount of the test sample shall be not less than 5 cc. from each bleeding irrespective of the volume of the blood drawn. This amount shall be cultured at 37° C. for 7 days in one or more portions of thioglycollate medium. If evidence of contamination appears the plasma shall be discarded.

"16. *Sterility test on the pool.*—When this method of testing for sterility is selected the procedure shall be as follows: For liquid or frozen plasma 2 separate samples shall be withdrawn from the well mixed pool for the sterility test. The size of each sample shall be not less than 20 cc. for each liter of plasma in the pool under test. For the sterility test the entire volume of one of the two samples shall be planted in one or more portions of thioglycollate medium. If evidence of contamination appears the test shall be repeated with the second sample and if this test also shows the presence of contamination the pool shall be discarded.

"18. *Sterility test on the final containers.*—When liquid plasma is being processed a sterility test shall be made on the plasma in a final container, but this test shall not be made before the lapse of at least two weeks since filling the containers from the plasma pool. During this interval the plasma shall have been stored as required under section 42.

"19. For the purpose of the sterility test a sample shall be withdrawn from the pool-reservoir at the time of filling the final containers. This sample shall be a pool made up of the first 25 cc. flowing from the plasma-reservoir and the last 25 cc., provided not more than 25 final containers are involved. If the plasma-reservoir is of larger volume, then additional samples shall be prepared on the basis of one addi-

tional sample for each 25 final containers or fraction, the sample to be withdrawn from the plasma-reservoir just prior to and immediately following each group of 25 final containers. The samples shall be drawn directly into a separate, empty, final container which has been selected at random from the stock of empty sterile containers.

"20. After the lapse of the 2-week storage period (sec. 18) and after allowing the sample to come in contact with the entire inside surface of the container, not less than a 20 cc. portion shall be withdrawn for planting for the sterility test. The dilution of the plasma in the culture medium shall be such that the preservative contained in the plasma will no longer prevent bacterial growth. (See appendix B, sec. IV.) In case contaminations appear in any tube planted, the test may be repeated from the unused portion of the sample, but no lot shall be passed until the final test shows no growth.

"21. However, if the lot fails because of growth in the above tests, a retest may be made by selecting at random one of the filled final containers represented by the original sample tested and by carrying out similar sterility tests. The absence of growth on this retest shall negate the previous unsatisfactory sterility tests and the final containers filled between the two portions of the sample showing contamination shall be released as satisfactory.

"22. *Frozen plasma.*—Proceed exactly as directed for liquid plasma (secs. 19, 20, and 21) except that the test sample shall be held for the 2-week period as directed in section 18 but in the frozen state as directed in section 31, along with its pool mates, before thawing as directed in section 46.

"THE FINAL CONTAINER

"35. It is recommended that either the label or an accompanying circular of instructions contain a statement warning against the danger of overheating the liquid plasma before administration and that when safe warming facilities are not available, or when emergency exists, it is safe to administer the plasma without preliminary warming.

"36. It is recommended that either the label or an accompanying circular of instructions contain a warning as to the danger of injecting the plasma intravenously without the use of a filter in the lumen of the tube leading from the plasma-reservoir to the recipient." (See appendix B, sec. VIII.)

"DATING AND STORAGE OF PLASMA

"41. *Liquid plasma.*—The expiration date for liquid plasma shall not exceed 1 year from the date of manufacture. The date of manufacture is calculated as the date of bleeding the donor.

"42. Liquid plasma shall be stored as near as possible at a constant temperature within the range 15° to 30° C. A statement to this effect shall appear on the label. This temperature range does not necessarily insure the preservation for the entire dating period of all immune bodies which may be present in normal plasma. If these substances are desired it is recommended that recourse be had to dried plasma, either liquid or dried normal human serum, or to liquid plasma within 2 months of processing or thawing.

"43. *Frozen plasma.*—The expiration date shall not exceed 3 years from the date of manufacture when kept continuously in the frozen state as specified in section 45. The date of manufacture is calculated as the date of bleeding the donor.

"44. Frozen plasma which has been thawed as in section 46 and stored as described in section 42 may be given an expiration date of not to exceed 1 year from the time of thawing.

"45. Frozen plasma shall be stored continuously at minus 18° C. or lower until needed.

"46. Frozen plasma shall be thawed in the following manner: The bottle of frozen plasma is placed immediately in a constant temperature water bath provided with circulating water, or its equivalent, and maintained at 37° C. As soon as all of the plasma is melted, and its temperature has reached the specified storage range for liquid plasma, the bottle is removed and stored until used as recommended for liquid plasma.

"47. It is recommended that frozen plasma be kept in the processing laboratory or other suitable depot and that shipments be made only after thawing as directed in section 46. *Provided, however*, where either the processing laboratory or the purchaser can assure satisfactory shipment in the frozen state and eventual thawing as described in section 46, this may be done."

Section IV

Fluid Thioglycollate Medium for the Sterility Test

"Full details of the four formulae of the fluid thioglycollate medium recognized as standard for the sterility testing of biologics is contained in a memorandum prepared by the National Institute of Health. The formulae of Brewer and Linden are recognized as equally satisfactory, but the Linden variation is recommended as easier to prepare and more economical.

Method B—Fluid Thioglycollate Medium (Linden)

	Grams
Peptone	20.0
Dextrose (anhydrous)	5.0
Yeast extract	2.0
Sodium thioglycollate	1.0
Sodium chloride	5.0
Agar (less than 15 percent moisture by weight)	.5
Dipotassium phosphate (K_2HPO_4)	2.5
	Cubic Centimeters
Distilled water	1,000.0
0.2 percent solution of Methylene blue (cert.)	1.0

"Dissolve the agar in half the volume of distilled water by boiling or heating in the Arnold. Dissolve the remaining ingredients, except the methylene blue, in the remaining water with the aid of heat. Now mix the two portions, adjust the reaction with sodium hydroxide to such a point as experience shows will result in a pH of 7.5 ± 0.1, in the completed and sterilized medium. Filter clear while hot and add the methylene blue solution. Distribute into final containers of the desired size and sterilize in the autoclave for 18–20 minutes at 15–17 pounds pressure (121° to 123° C.).

"A medium may be prepared as a premixed dehydrated stock of the essential ingredients contained in method B formula. Such premixed stock is now being commercially prepared and this has proven equally satisfactory and has the advantage of being certified as to growth qualities. Directions for preparation are given on the label.

"Experience has shown that 7.5 cc. of this culture medium will neutralize the bacteriostatic action of that amount of mercury present in 1 cc. of inoculum which has been preserved with 1:10,000 phenyl mercuric borate, or its mercurial equivalent, provided the inoculum is well mixed in the medium.[9]

"At the end of the incubation period used for the sterility test less than 50 per cent of the medium in each tube shall have changed from the color of the fresh medium to a green color."

Section V

Safety Test

"A test for safety, which means the absence of toxic substances as indicated by animal inoculation, should be made on each lot of plasma. The plasma sample for this test in the case of liquid or frozen plasma should be the last portion removed from the pool bottle at the time of

[9] It has been shown that 5 cc. of medium is sufficient to neutralize the bacteriostatic action of mercury in 1 cc. of plasma containing 1/10,000 merthiolate.

filling the final containers. With dried plasma the safety test is made on a portion of the dried sample taken for the sterility test.

"The safety test is made by injecting either 0.5 cc. intraperitoneally into a mouse, 5 cc. into a guinea pig, or 25 cc. into a rabbit. Observation should be for at least 5 days."

Section VI

A Method for the Determination of Residual Moisture

"1. The amount of residual moisture remaining in plasma which is labeled as a dried or desiccated product shall contain not more than 1 percent moisture when determined by the following method: Expose a 1 to 2 gram sample of the plasma, evenly distributed in a weighing bottle not less than 50 mm. in diameter in a vacuum desiccator at less than 1 mm. pressure and over fresh phosphorus pentoxid at room temperature until the weight remains constant to the third decimal."

Section VII

Pyrogen Test

"*Test animal.*—Use healthy rabbits weighing 1,000 gm. or more which have been maintained for at least 1 week on a uniform diet and have not lost weight. Test the thermometer to determine the time required to reach maximum temperature. If the animals have not been previously used for such tests, take four rectal temperature readings on each of the animals at 2-hour intervals 1 to 3 days before use. Insert the thermometer beyond the internal sphincter, and allow it to remain sufficient time to reach maximum temperature, but in no case less than 90 seconds, before the reading is recorded. Discard those animals with a temperature in excess of 39.8° C. On the day of the test take a control temperature reading before the injection of the test material. Animals may be used for the test and in subsequent tests after a rest period of not less than 2 days, provided the control temperature reading taken on the day of the test does not exceed 39.8° C. The reading taken on the test day constitutes the normal temperature of the test animal from which a subsequent rise due to the injection of the test material is calculated. Keep test animals in individual cages protected from disturbances likely to cause excitement. Exercise particular care to avoid exciting the animals on the day of taking the control temperatures and on the test day.

Withhold food from any animal used, beginning 1 hour before the first temperature reading, and permit no food until the day's record is completed. Free access to water is allowed. Keep the animals at uniform temperatures (±5° C.) during the control and test period. They should preferably be housed in quarters maintained at constant temperature and humidity.

"*Conduct of the Test.*—Warm the product to be tested to approximately 37° C. and inject 10 cc. per kgm. of rabbit, intravenously through the marginal ear vein within 15 minutes subsequent to the control temperature reading on the day of the test. Record the temperature 1 hour subsequent to the injection and each hour thereafter until three recordings have been made. Syringes and needles used for these injections must have been treated to render them pyrogen-free and then sterilized. Not less than five rabbits shall be used for each test and the test shall be considered positive if three or more of the five animals show an individual rise in temperature of 0.6° C. or more above the normal established for each of these animals. If only one or two of the five animals show a positive response, the test must be repeated on a second group of five additional animals. The test shall be considered positive if two of the second group of five animals show an individual rise in temperature of 0.6° C. or more above the normal established for these animals."

Section VIII

A Filter Adequate for Removal of Particulate Matter

"1. A filter adequate for the removal of all particles of such coarseness as to be dangerous for intravenous administration must be placed in the tube through which the plasma flows from the plasma-pool reservoir to the final container. In addition, it must be placed in the lumen of the tube provided with each unit of either finished liquid, frozen, or dried plasma for the purpose of transferring the plasma from the container to the blood stream of the recipient. Further, if such equipment is not provided, the label on the final container or the accompanying direction circular for administration of the plasma must bear a warning that such filter must be used.

"2. A filter shall be considered adequate provided it is no less effective than is obtained by glass tape when prepared as follows: A piece of glass tape 3 inches long, ¾ inch wide and having 32 picks and 32 ends per inch is folded double cross-wise and then folded loosely on the long axis. It is then loosely inserted into a glass tube having a slight constriction at one end and having an inside diameter com-

parable to the inside diameter of the rubber tube used. The folded end of the glass tape is *always placed* toward the constricted end of the glass tube and the glass tube is *always placed* with the constricted end towards the final container in filling or the recipient in administering the plasma intravenously. This prevents shreds of glass tape from entering the filtered plasma and also prevents the glass tape from being drawn down into the rubber tube."

Section IX

N. I. H. Requirements for Commercial Firms

In this section are given several requirements of the National Institute of Health in addition to those given previously (p. 44) which apply specifically to commercial firms and to the preparation of dried plasma, but do not necessarily apply to hospital practice.

1. The drawn blood (citrated) must be placed at 2° to 5° C. within 1 hour of bleeding, must be freed of its cells within 72 hours, and if the plasma or serum is to be held in the frozen state or be dried, it must be brought to the frozen state within 72 hours.

2. The plasma from a minimum of eight bleedings, which has been obtained by centrifugation in the original bleeding bottles, must be pooled and well mixed before the final containers are filled in order to adequately dilute the iso-agglutinins and also to equalize the protein content.

3. The general practice is to discard plasma showing an appreciable amount of hemoglobin; 25 mg. percent is the maximum allowed.

4. A satisfactory preservative is added to the pool and thoroughly mixed prior to the distribution of the plasma into the final containers. (At the time of this writing 1:15,000 phenyl mercuric borate or phenyl mercuric nitrate, or 1:10,000 merthiolate are considered satisfactory preservatives.)

5. If the final product is processed to the dried state, the finished product must contain not more than 1 percent of moisture, the container must be flame sealed, or its equivalent, under vacuum, in a container of good quality glass and so constructed as to permit the vacuum to draw the diluent into the container, thereby permitting solution of the dried product without opening the container or releasing the vacuum.

6. Explicit instructions for thawing frozen plasma must accompany each unit of material, preferably as a direction on the label. The direction is: Thaw rapidly by immersing the bottle of frozen plasma, immediately upon its removal from refrigeration, in a water bath main-

tained constantly at 37° C., until the plasma has become liquid and has reached room temperature. Administration should follow promptly after the plasma has reached room temperature (25° C. or approximately 80° F.).

7. Similar explicit instructions should accompany each unit of dried plasma. Besides a description of the method to be used for the solution of the dried plasma, the instructions should also specify that a pyrogen-free, sterile, and otherwise suitable diluent must be used. (See Pyrogen Test.)

Section X

U. S. P. Requirements for Citrated Normal Human Plasma

The United States Pharmacopoeia (U. S. P. XII) recognizes human blood plasma in three different physical forms as being adequate for intravenous administration; i. e., liquid plasma, frozen plasma, and dried plasma. The description provided by U. S. P. XII is reproduced herewith in full:

"Citrated normal human plasma is the sterile plasma obtained by pooling approximately equal amounts of the liquid portion of citrated whole blood from eight or more humans (Homo sapiens) who have been certified by a qualified doctor of medicine as free from any disease which is transmissible by blood transfusion at the time of drawing the blood.

"Each bleeding is drawn under aseptic precautions into individual sterile centrifuge bottles already containing sterile, physiological solution of sodium citrate (4 percent dihydric sodium citrate) in the proportion of 50 cc. per 500 cc. of whole blood. The plasma is separated by centrifugation and transferred to a pool by means of a closed system. Sterility tests are made, a preservative is added, and the plasma is distributed into final containers through a closed system. Citrated normal human plasma complies with the requirements of the National Institute of Health of the United States Public Health Service.

"*Description.*—Citrated normal human plasma may be dispensed as liquid plasma, as frozen plasma, or as dried plasma. Citrated normal plasma must be free from harmful substances detectable by animal inoculation, or by other means, and must not contain an excessive amount of preservative.

"(a) *Liquid plasma.*—Freshly collected citrated human plasma is a slightly opalescent liquid of a faint yellowish or amber color and practically odorless in the absence of a preservative possessing an

odor; it contains no visible particles and is free of cellular elements save for a variable number of blood platelets. Increased opalescence or a precipitate of fibrin may develop on standing. As a stabilizing agent not more than 10 percent of dextrose in the form of Injecto Dextrosi (50 percent dextrose) may be added.

"(b) *Frozen plasma.*—This is liquid plasma, without added dextrose, frozen quickly by rotating the container properly inclined while partially immersed in a freezing bath within 72 hours of bleeding. It is imperative that frozen plasma be kept continuously in the frozen state until required for use, then liquefied by placing in a water bath at 37° C. and administered promptly.

"(c) *Dried plasma.*—This is frozen plasma which has been dried from the frozen state under vacuum; it contains not more than 1 percent moisture as determined by exposing a 1–2 gram sample, evenly distributed in a weighing bottle not less than 60 mm. in diameter in a vacuum desiccator at less than 1 mm. pressure over fresh phosphorus pentoxide at room temperature until the weight remains constant to the third decimal. It has a light yellow to deep cream color, is microscopically of a honeycomblike structure, and shows no evidence of fusion.

"*Regulations.*—The outside label must bear the name 'Citrated Normal Human Plasma' and indicate the volume of original normal human plasma represented in the container, the manufacturer's lot number of the plasma, the name, address, and the license number of the manufacturer, and the date beyond which the quality of the contents may not be maintained.

"*Storage.*—Preserve liquid plasma at a temperature between 10° and 20° C., frozen plasma at a temperature between minus 5° and minus 20° C. Dried plasma shall not be exposed to excessive heat. Citrated Normal Human Plasma must be dispensed in the unopened glass container in which it was placed by the manufacturer."

APPENDIX C

Treatment of Shock and Burns with Citrated Plasma

It is beyond the scope of this manual to discuss extensively the therapeutic uses of citrated plasma (see p. 6.) It seems desirable, however, to give a brief review of the use and dosage in shock accompanying those clinical conditions most commonly occurring in civil and military emergencies.

Any evaluation of the fluid replacement therapy in shock and burns must consider the extent and degree of the lesion, the age and physical status of the patient, as well as other therapeutic measures undertaken. It is well known that minimal trauma may produce serious systemic reactions in the physically debilitated. In addition, the state of hydration of the patient must be estimated as accurately as possible. This is not always easy under emergency conditions, since laboratory studies may of necessity be deferred.

In the treatment of shock all that is possible must be done to prevent the initiating factors from acting a sufficiently long time to produce clinical manifestations. The best treatment is, in other words, prevention. *Patients exposed to obvious and sufficient precipitating factors must be treated as potential cases of shock without waiting for the appearance of clinical symptoms.* Thus, a patient who has sustained extensive injury with crushing of tissues, with or without evident blood loss, should not be submitted to an extensive operative procedure involving general anesthesia without a dose of 17.5 to 35 grams of plasma proteins (250 to 500 cc. of undiluted plasma).

Patients in shock, with such manifestations as cold, moist skin, grayish-blue color, feeble and rapid pulse, blood pressure unchanged or slightly lowered, must be treated immediately and adequately. The management of early shock is as a rule a simple and successful procedure, whereas late shock is often very difficult to combat. This line of demarcation between early shock and late shock divides the patients who can be successfully treated with relatively small doses (17 to a maximum of about 54 grams, or 250 to 750 cc. of undiluted plasma) and those in whom larger doses (54 to 106 grams of plasma

proteins, or 750 to 1,500 cc. or more of undiluted plasma) must be employed repeatedly with only a fair chance of success.

The severe forms of shock are usually present in patients who, regardless of the severity or nature of the initiating factors, have been allowed to go for a period of time without adequate treatment. As a typical finding, they show considerable drop in the total amount of plasma proteins. These patients usually have also a conspicuous drop in the blood pressure and particularly in the pulse pressure, a rapid thready pulse, severe reduction of the skin temperature, collapsed veins, slow flow of blood from wounds, thirst, and low urinary output.[10] In these cases, maximum doses of plasma must be given (105 to 210 grams of plasma proteins, or 1,500 to 3,000 cc. of undiluted plasma and even larger doses). The first 250 to 500 cc. should be given without delay. Difficulty may be experienced in finding a suitable vein under these extreme conditions. To await a drop in the blood pressure before making a diagnosis of shock is reprehensible, but in the treatment of shock it is a very good index by which to judge the efficiency and adequacy of treatment in general and the dosage of plasma in particular.

Patients with burns require very large amounts of plasma and must be watched carefully for the first few days if shock is to be avoided. A good general rule is that 1,000 cc. of whole (undiluted) plasma for every 10 percent of body surface burned is required during the first 24 hours. Almost as much may be needed on the second day. *The use of large quantities of plasma (2,000 cc. or more within 24 hours) may at times result in the development of pulmonary edema, particularly after the inhalation of fumes or in the presence of chest injury.*

In the treatment of patients showing evidence of impairment of renal function, the presence of a mercurial preservative in plasma should be borne in mind, since it may conceivably add to renal damage if more than 2,000 to 3,000 cc. in plasma are administered in the course of 24 hours. Ordinarily, the speed of intravenous administration of plasma should not exceed 150 to 300 drops (10 to 20 cc.) per minute. *In advanced shock, where time is at a premium, it should be given as rapidly as possible—even to the extent of using two intravenous routes at the same time.* The reader is referred to OCD Publication 2212, "The Clinical Recognition and Treatment of Shock," for a more detailed discussion.

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[10] Hemoconcentration may be present in severely dehydrated patients and in patients with severe burns, or crush and abdominal injuries. Hemodilution is usually present in hemorrhage and skeletal trauma (which implies hemorrhage).

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